GALVANOTAXIS AND CHEMOTAXIS OF CILIATE INFUSORIA. Part I.

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CONTENTS.

I. Historical and introductory.

THE experiments to be described in this paper were made with the object of obtaining evidence as to the nature of the phenomena known as galvanotaxis or galvanotropism. I have confined myself to observations on the ciliate Infusoria, and do not propose in the present paper to enter into the question of how far the superficially similar reactions of certain metazoa have an ultimate origin of the same nature.

The earliest experiments on the effect of the current on unicellular organisms were made by Kühne[1], who, using platinum electrodes, passed a constant current through water containing Actinosphærium. The apparent effect was to cause a stimulus on the anodic side of the animal during the whole time of passage of the current, and a similar but weaker stimulus on the kathodic side after break of the circuit. With stronger current a weaker effect appeared on the kathodic side during passage of the current, and this he attributed to "voluntary" contraction. Verworn[2], in 1889, repeated and confirmed Kühne's observations, using non-polarisable electrodes, and extended them to other Rhizopods. He also first observed that directive effect of the current on free-swimming infusoria which forms the subject of this

paper. He found that Paramœcium, for example, turned, at make of the current, with its anterior end to the kathode and swam, with its long axis always placed along a line of current, to that pole. The other ciliate Infusoria examined behaved in a very similar manner, with the exception of Opalina, which moved always to the anode, and Coleps hirtus, which was attracted sometimes to the kathode, sometimes to the anode.

The flagellates examined collected at the anode, with the exception of Trachelomonas and Peridinium, which, like the ciliates, went to the kathode. With very strong currents he observed a shrinkage and ultimate disintegration at the anode-pointing end of Paramœcium, while, in Opalina, a disintegration of a different kind was seen on the kathodic side. He concluded, therefore, that the motion to one pole was caused by a stimulus on the side of the other pole, comparable to the stimulus observed in Rhizopods. The suggestion that the kataphoric effect of the current might play some part in the phenomena was dismissed by Verworn on the grounds that dead or anæsthetised animals showed no such reaction.

Ludloff[3], working with Paramœcium, found that the rate of collection at the kathode was not proportional to the strength of the current; it rose rapidly to a maximum, with increasing strength of current, and then diminished. He concluded that the effect was physiological and not purely physical. The orientation of the organisms in the lines of current was explained by the action of the cilia, which he observed to strike forwards on the kathodic, backwards on the anodic side of the animal. To account for the passage to the kathode, he supposed that the backward striking cilia at the anodic end of the animal worked more powerfully than the forward-striking cilia at the kathodic end.

Loeb and Budget[4], assuming the correctness of Verworn's theory of anodic stimulation, attempt to bring the result into harmony with Pflüger's Law of Stimulation. They suppose that alkali is set free where the current leaves the water and enters the animal, acid where it leaves the animal to enter the water. Their evidence is the supposed similarity between the effect of a strong current at the anodic end and that of immersing the animals in a 1 % solution of caustic soda.

Mouton[5] discusses the possibility of the action of the current being chemotactic in nature, and due to the elimination of acid and alkali at the poles. Using metal electrodes of a special shape, he concludes from the results obtained that the action is not of this

nature. He found that Colpidium swam towards the metallic kathode, but, being repelled by the alkali there eliminated, formed a widening ring round the electrode. Up to this point the tendency has been, then, to regard the phenomena as entirely of physiological origin. More recent papers have given greater prominence to the possibility of a physical origin.

Birukoff[6], using repeated induction shocks as the stimulating current, and applying them through variously shaped electrodes of tin-foil to the animals contained in a thin layer of water under a cover-slip, finds that the collection at the kathode can be imitated by the substitution of inert particles, such as grains of carmine, for the living organisms. The behaviour of the latter differs only in that they swim to the points of least current density, while the particles collect where the density of current is at a maximum. He attributes then the movement to the kathode entirely to the kataphoric stream produced by the current in the water, regarding the movement from points of greater to those of less current density as the only physiological element in the phenomena.

Carlgren[7] observed the effect of currents on Volvox aureus. In this colonial flagellate he observed a setting of the long axis of the colony in the lines of current and a movement to the kathode. During prolonged passage of the current they move away from the kathode and often form a secondary collection at the anode. The flagella on the anodic side were inhibited, on the kathodic side unaffected. In fixed individuals he observed a shrinkage on the anodic and a swelling on the kathodic side, with migration of the parthenogonidia towards the anodic side. These effects he reproduced on dead specimens by the use of strong currents. He also reproduced, on dead Paramœcia, Colpidia and Amœbæ, the anodic shrinkage and kathodic swelling obtained by Verworn in living specimens, and by him attributed to stimulation. These effects were only seen in water, and are obviously due to the kataphoric action of the current — the "electrical endosmose" of Wiedemann, Porret, Quincke and others. He concludes that this physical action of the current accounts for many of the supposed stimulation-effects on Rhizopods, and that it probably plays a part in galvanotaxis.

Pearl[8] again investigates the behaviour of the cilia in galvanotaxis. He confirms Ludloff's observations and finds in addition that the "forced movement" of the cilia, which causes "setting," is preceded by a "motor reflex" of the type described by Jennings[8a] as the

result of mechanical, chemical or thermal stimuli. He concludes that chemotaxis to liberated ions plays a small part, if any, in galvanotaxis, the forced movement being due to a special electrical stimulus, the nature of which is unknown. The tendency of Paramœcium to recede from the kathode when currents of great strength are used may, he considers, be due to the anaphoric action of the current on particles.

It is evident that, even among those observers who consider that purely physical elements are present in the so-called galvanotaxis, there is no concurrence of opinion as to which particular elements are of physical, which of physiological origin.

Most of my own experiments have been made with the infusoria parasitic in the intestine of the common frog. My attention was directed to them in the first place by the anomalous results obtained by Verworn in his experiments on Opalina and Nyctotherus. Passing a strong current through a watch-glass containing these animals in 0·6 % salt solution, he found that Opalina collected at the anode, Nyctotherus at the kathode. Such a difference was left quite unaccounted for by the suggestion, made by various observers, that the anode-seeking habit of Opalina might be due to the fact that it was examined in physiological saline, whilst other forms had been tested in more or less pure water. The comparatively large size of these animals, and of the three species of Balantidium frequently found in the intestine of Rana temporaria, makes them convenient objects for the study of galvanotaxis. The fact that their normal habitat is a medium containing a considerable proportion of salts necessitates their examination in a solution containing from ·5 %—·75 % of sodium chloride, since most of them die rapidly in water. This, however, is in itself a conspicuous advantage, since it minimises those physical effects which have been considered chief or important factors in galvanotaxis.

Faraday[9] found that the electrification of substances by contact with water was abolished by the addition to the water of small quantities of soluble salts, acids, etc., which lessened its electrical resistance. This contact electrification has been considered by Quincke[10] to be the basis of the kataphoric action of the current on water in glass tubes or porous non-conductors, and of its anaphoric action on suspended particles: and in accordance with this view he found that addition of ·1 % of sodium chloride of ·04 % of sulphuric acid to the distilled water completely abolished the phenomena in question.

Jürgensen[11] and Weyl[12] also found that the similar behaviour of suspended particles which they described was abolished by the

addition to the water of substances increasing its conductivity. Hardy[13] likewise finds that the kataphoric and anaphoric action of the current on suspended colloid particles of derived albumins decreases with increase in the conductivity of the solutions. One may, therefore, expect the physical element of galvanotaxis to be, at any rate, very small when such a medium as physiological saline is substituted for the more or less pure water in which most of the experiments have hitherto been made. In order to determine the nature of the still striking reaction to the current which these animals show in such a solution of comparatively great electrical conductivity, a comparative examination was made of the chemotactic reactions of the five species mentioned above to acids and alkalis, and of their response to the constant current. The modifications produced in either response by treatment of the organisms with salt-solutions of varying reaction were tested. Other experiments were made on the changes produced in the galvanotaxis by varying the concentration of the medium, and the action of the cilia when under the influence of the current was examined in the cases of Opalina and Nyctotherus.

II. Note on the species examined, and their distribution in the intestine of the frog.

At the outset of my experiments I observed some peculiarities, which seem worth notice, in the distribution of the various species of Infusoria in the frog's intestine. The species of ciliata which I have found in the intestine of Rana temporaria are five in number and are distributed as follows:

I. *Balantidium duodeni* (Stein). For description *see* Saville-Kent[14], *Manual of the Infusoria*, Vol. II. p. 78. This species was described by Stein in Rana esculenta as confined to the duodenum. I have found it repeatedly in R. temporaria, in which species it is also confined to the duodenum when in its normal condition. In autumn and winter frogs, in which it was often extraordinarily abundant, it was often present, in a condition which was probably an early stage of encystment, in the lower parts of the intestine. In this condition the animal was practically spherical; no trace of peristome could be seen, and the movement was chiefly rotation on its axis caused by slowly beating marginal cilia. The same condition was produced artificially by an accident, the normal forms being left all night in markedly acid

salt-solution, and being found in the spherical condition when examined on the next day.

Balantidium elongatum (Stein). *See* Saville-Kent, Vol. II. p. 577.

The form which I have referred to in this paper as Balantidium elongatum corresponds, as far as I have been able to observe, in all details to Saville-Kent's description of that species, except in that the nucleus has the form of a long thin rod, bent into the form of a horse-shoe. If the "ovate endoplast" is a sound diagnostic character the animal here dealt with must be of a different species. There can, at the same time, be no doubt as to its distinctness from B. entozoon (*infra*). Its general form corresponds to the description of B. elongatum, while its chemotactic and galvanotactic reactions are markedly different from those of B. entozoon, as is also its distribution in the intestine.

In the absence of any completely corresponding description it will be referred to as Balantidium elongatum, which was described by Stein in the intestine of Triton tæniatus and Rana esculenta. I have found the species, often in great abundance, in R. temporaria, in which it shows a marked tendency to accumulate in a zone about the junction of the small intestine proper with the duodenum. When abundant it may be found in this region in dense swarms, and in much smaller numbers in the rest of the small intestine, extending to the junction of the latter with the rectum. In two cases, in which B. duodeni was absent, B. elongatum was found in small numbers right up to the pylorus. Normally, however, it does not extend above the lower third of the duodenum.

This form and B. duodeni were found in great abundance in the late autumn and early winter months. From all the frogs examined in February, March and April, B. duodeni had disappeared, and during the latter part of those months B. elongatum as well, B. entozoon being at the same time rare. More recently, in May, June, July and August, all the species have been found again, but not in abundance comparable to that of the autumn. The frogs examined were from the common stock in use in the laboratory, and collected from various localities round Cambridge, so that it seems hardly possible to suppose that the presence of these species is a local characteristic. It seems more probable that the disappearance in question is due to a coincidence between the reproductive period of these species with the copulation and spawning-time of the host. If that is so, an explanation of the coincidence may possibly be found in the marked emptiness of the small intestine at this period, and an examination of the variations of

intestinal reaction, in the frog, with the time of year might furnish a clue. This, however, I have not yet been able to make.

Balantidium entozoon (Ehrenberg). *See* Saville-Kent, Vol. II. p. 577. This smaller species occurs, mingled with Opalina and Nyctotherus, at the junction of the small intestine with the rectum.

Nyctotherus cordiformis. *See* Saville-Kent, Vol. II. p. 580. This species of Nyctotherus occurs with Opalina and Balantidium entozoon at the junction of the small intestine and rectum. Its abundance varies greatly, but it is seldom so abundant as Opalina. It tends to accumulate in the extreme upper end of the rectum, and does not extend so far into the small intestine as does Balantidium entozoon, nor so far down into the rectum as does Opalina.

Opalina ranarum. *See* Saville-Kent, Vol. II. p. 559. Opalina is found in most frogs accumulated in great numbers at the upper end of the rectum. As mentioned above it usually extends rather further down into the fæcal mass in the rectum than does Nyctotherus. We find, then, in this zone comprising the lower end of the small intestine and the upper end of the rectum three distinct organisms; and it can further be detected in some specimens that each has a zone of maximum abundance peculiar to itself, that of B. entozoon being the highest, that of Nyctotherus in the middle, and that of Opalina the lowest. For the greater part of their extent, however, these three zones overlap, so that in most parts of this ileo-rectal zone there will be found a mixture of the three forms when present.

In the following table are arranged the results of observations on 16 freshly-killed frogs, examined in November. The intestine was cut into the following parts:

(1) Upper half of duodenum.
(2) Lower half of duodenum.
(3) Upper half of ileum.
(4) Lower half of ileum.
(5) Rectum, with a very short length of ileum attached.

Each was opened separately in a watch-glass filled with ·6 % saline, and the contents agitated with the solution. Each was then carefully examined with a low power ($\frac{2}{3}$ in. obj.) of the microscope.

The following abbreviations are used: B. duod. = Balantidium duodeni in the normal state; B. duod (sph.) = Balantidium duodeni in the early stage of encystment described above; B. elong. = Balantidium elongatum; B. entoz. = Balantidium entozoon; Nyct. = Nyctotherus; Op. = Opalina.

	DUODENUM		SMALL INTESTINE		RECTUM
	Upper	*Lower*	*Upper*	*Lower*	
1.	B. duod.	B. elong. and duod.	B. elong. and duod. (sph.)	B. duod. (sph.) & entoz. (few)	Op. Nyct. B. entoz. and B. duod. (sph.)
2.	B. elong.	B. elong. (great number)	B. elong.	0	Op. Nyct. B. entoz. and elong. (few)
3.	0	B. elong. (great number)	B. elong.	Nyct. (very few)	Op. and Nyct. (great number)
4.	B. duod.	B. duod. and elong.	B. elong.	0	Op. Nyct. & B. entoz.
5.	B. duod.	B. duod. and elong.	B. elong.	B. entoz.	Op. Nyct. B. entoz. and duod. (sph.)
6.	B. duod.	B. duod. and elong.	B. elong.	B. elong. (few) & duod. (sph.)	Op. Nyct. B. entoz. and B. duod. (sph.)
7.	0	B. elong.	B. elong.	0	Op. Nyct. B. entoz. and elong. (few)
8.	0	0	0	0	Op. Nyct. & B. entoz.
9.	B. duod.	B. duod. and elong. (few)	B. duod. (sph.)	B. duod. (sph.) Nyct. B. entoz. (few)	Nyct. and B. entoz.
10.	B. duod.	B. duod. and elong.	B. elong. and duod. (sph.)	B. elong. and entoz.	B. entoz.
11.	B. elong.	B. elong. (great number)	B. elong.	B. elong. and op. (few)	Op. and B. elong. (few)
12.	B. duod.	B. duod. and elong.	B. elong.	B. elong. (few)	Op. Nyct. B. entoz. and elong. (few)
13.	B. duod.	B. duod.	B. duod. (sph.)	B. duod. (sph.)	Op. Nyct. B. entoz. and duod. (sph.)
14.	B. duod.	B. duod. and elong.	B. elong. and duod. (sph.)	B. entoz. and elong.	B. entoz.
15.	0	0	0	0	Nyct. and B. entoz. (few)
16.	0	B. elong.	B. elong.	B. elong. and Op. (few)	Op. B. entoz. and B. elong.

In a few cases a more exact examination of the distribution was made. In No. 6, for example, the lower end of the duodenum and a short length of ileum continuous with it were cut into a series of short pieces 1 cm., or less, in length. Four successive sections of this kind—the last 3 of the duodenum and the 1st of the ileum—gave the following yields of parasitic ciliates :—

(1) Balantidium duodeni only.

(2) Balantidium duodeni with a few elongatum.

(3) Balantidium elongatum abundant, with a few duodeni.

(4) Balantidium elongatum, among which only 2 duodeni were found.

Examples (2) and (11) in the table show that, when B. duodeni is absent and elongatum very abundant, the latter may be found along the whole length of the duodenum, while several instances are given of its extension even to the rectum.

It would be of considerable interest to determine whether this distribution is accounted for by variations of intestinal reaction, and

accords with the differences of chemotactic response detailed below. It is clear that the consistency of the fæcal food-matter cannot be held to account for the phenomenon, since we find the three species of Balantidium, resembling one another in the possession of a rudimentary mouth, showing the widest differences in distribution, while in the rectum, where the fæces have usually become firm, are found a Nyctotherus (with complete mouth, pharynx, rectum and anus), a Balantidium (with rudimentary mouth only), and an Opalina, which has no trace of mouth or anus and appears to be entirely saprophytic. A few experiments were made on the reaction of the intestinal contents, but it would hardly be legitimate to apply results obtained in the absence of several species to an explanation of the distribution of those species when present. It may be mentioned that the results obtained seemed to indicate a possibility of explaining the distribution in connexion with chemotactic reactions, but further examination of the question has been postponed until frogs can again be obtained in which all the species are abundant.

III. Comparison of chemotaxis and galvanotaxis.

For these experiments the organisms were shaken into the various salt-solutions in watch-glasses and were left in the solutions for times varying with their nature.

My early experiments were made in the ·6 % solution of crude salt in use as physiological saline in the laboratory. Very consistent results were obtained when the animals had been left in it for a sufficient time —half-an-hour or more—before the experiment was made. Certain discrepancies observed when the animals were freshly shaken into the solution and the experiment made at once, and particularly in cases where they were obtained from freshly-killed frogs, suggested to me that the reaction of the medium in which the animal had been living before the experiment had an effect on both chemotaxis and galvanotaxis. It was found that the laboratory salt solution was very distinctly alkaline to litmus or methyl orange, and gave a faint pink colour even with phenol-phthalein. Experiments were therefore made on specimens which were left for varying times in neutral, alkaline and acid salt solutions, and in this way completely consistent results were obtained. The animals were shaken into a neutral or faintly alkaline salt solution coloured with litmus. The intestinal contents have a reaction which varies with the freshness of the frog. In freshly-killed

frogs, for example, the rectal contents are usually faintly acid, but when taken, as was the case in a large number of experiments, from frogs which had been used for other purposes and had been dead in the laboratory for some hours, they were usually markedly alkaline. The reaction of the solution, therefore, was adjusted after the admixture with it of the intestinal contents containing the infusoria. In most cases the fæcal matter could be washed away by repeatedly drawing off the solution with a pipette and adding fresh quantities, since these infusoria all sink with comparative rapidity to the bottom of the solution. When a preparation in moderately clean and neutral salt solution had thus been obtained the reaction was finally adjusted to the required condition by the addition of ·6 % salt solutions containing small proportions of sulphuric or acetic acid, caustic soda or sodium carbonate. Since alkalinity when present seemed to be due to carbonates the addition of acid was accompanied by a vigorous stirring of the solution by a stream of air. A solution in which litmus took a purple tint unchanged by a stream of air blown through it, or by standing for several hours, was considered to be neutral; but it must be mentioned that small variations of reaction were doubtless present in the various solutions to which the description neutral was applied, owing to the fact that the colour of litmus changes not suddenly but by imperceptible gradations with change of reaction. With regard to the degrees of alkalinity and acidity described as "faintly" or "markedly" alkaline or acid it should be pointed out that it is impossible to make an exact estimate of the alkalinity or acidity of a solution which is crowded with organisms constantly affecting the reaction of the solution in their immediate neighbourhood.

It may be stated that solutions are described as "faintly" alkaline which, while of weaker alkalinity than the laboratory saline, gave a distinct blue colour with litmus. By a markedly alkaline solution is meant one containing ·005 %—·02 % of NaOH, while between these two may be reckoned the "moderately" alkaline reaction of the laboratory saline. A solution is described as "faintly" acid when it gives a distinct but slightly bluish red with litmus, as "markedly" acid when the reaction is a clear scarlet—as when it contains ·005 % H_2SO_4. By using sufficient bulk of these solutions the effect of local alterations of reaction may be so minimised as to be almost negligible, but it was considered that the fact of the existence of such changes made it unjustifiable to describe the reactions in more definite terms than those employed.

Experiments were tried with the use of a ·6% solution of pure sodium chloride as neutral saline. It was found, however, that the organisms died sooner in this than in laboratory saline neutralised, while boiling, with hydrochloric acid. It is the latter solution, therefore, which is here referred to as "neutral saline."

The test-solutions for chemotaxis were made up with neutral saline so that the effects might not be complicated by the effect of distilled water, which is rapidly fatal to these animals.

For the chemotactic experiments a drop of solution containing the organisms was placed on a large glass slide and covered by a large slip supported on short lengths of capillary tube. The test-solutions were drawn into rather narrower capillaries, the ends of which were passed under the coverslip into the drop (Fig. 1). Solutions were generally used in pairs, one capillary containing an acid salt solution, the other an alkaline solution, carefully made *equivalent*. The slide so prepared was placed on a black tile for observation with a hand-lens, and could be removed to the stage of a microscope for more careful inspection with a low-power objective.

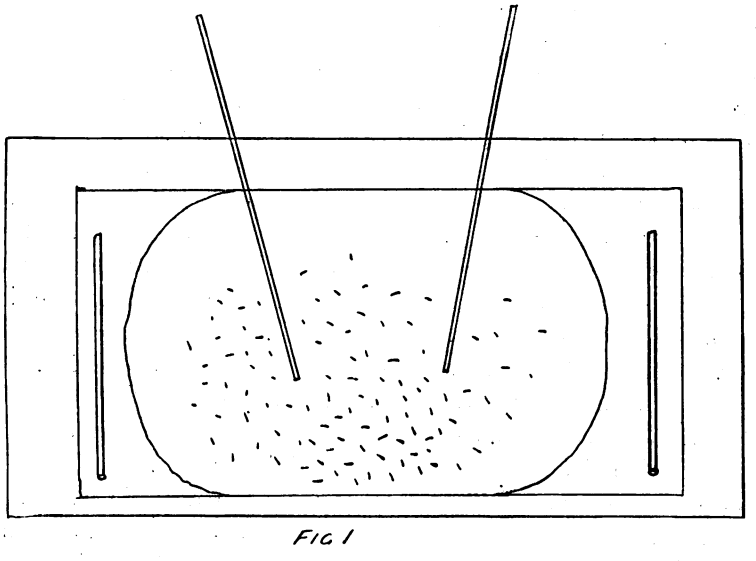


FIG 1

Fig. 1. Method of testing chemotaxis.

Another method, which gives more satisfactory results in some instances, is to insert the ends of the capillaries containing the test-solutions into a collection of the organisms at the bottom of a

watch-glass of salt solution, removing the tubes after a suitable interval for examination with the microscope.

In the use of either of the above methods the small calibre of the capillaries made rapid diffusion impossible, so that it was possible to watch the local effect of an acid or alkaline solution independently of that of the antagonistic solution used with it. To make it certain, however, that there was no confusion of effects—that, for example, a result attributed to an attractive effect of an alkaline solution was not in reality due to the repulsive effect of an acid solution used simultaneously—numerous control experiments were made with the use of one solution only. In most cases it was considered desirable to use the solutions in equivalent pairs so that the reaction of the general medium might change as little as possible during an experiment.

Galvanotactic reactions were tested in a stimulation-trough similar to that devised by Verworn. Two oblong slips of unglazed earthenware were cemented on to a large glass slide by means of as small a quantity of sealing-wax as would form a thin, even film between the earthenware and the glass. They were fixed parallel to one another at a distance of about 1 cm. and the cell was completed by side-walls of sealing-wax. By grinding with a wet stone the walls of the cell were levelled and its depth reduced to about 1 mm. To the ends of the slide were fixed wire holders for non-polarisable brush-electrodes, the brushes of which were applied to the porcelain slips. Before making an experiment the slide, with the electrodes in position, was placed in a dish into which was poured a thin layer of the particular salt solution to be used in the experiment. In this it was left for some minutes, so that earthenware and brushes might be saturated with the solution. In the absence of this precaution the effects of the current were liable to be complicated by chemotactic phenomena.

Fig. 2 shows the trough with the electrodes in position. The current was furnished by a battery of 12 bichromate 1 pint bottle-cells, usually arranged so that current from 3, 6, 9 or 12 cells could be used, and its direction changed by means of a Pohl's reverser.

The object of the experiments being a comparison, in the case of each animal, of the chemotactic and galvanotactic reactions under varying circumstances each of the organisms will be dealt with separately and the various phenomena will be presented in such order as to facilitate the comparison. In the actual experiments it was, in the case of the first three animals, a rare occurrence that any one species was examined separately, since in most cases the three are

found intermingled at the junction of the small and the large intestine. Their reactions, however, will be described separately except when combined description is necessary for comparison.

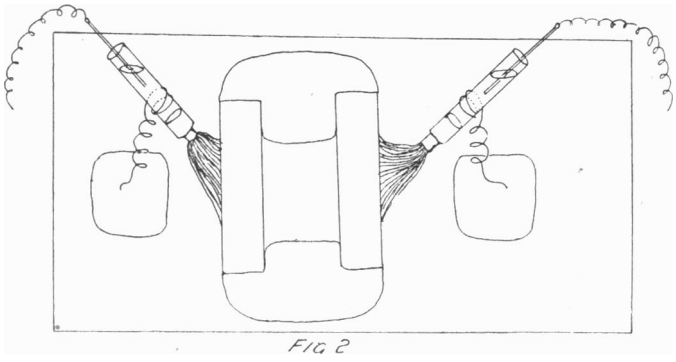


Fig. 2. Diagram of trough with electrodes.

Opalina Ranarum.

Opalina is much more delicate and more easily affected by solutions than are the other genera here dealt with. This is probably due to its flattened form, on account of which it presents to the action of the solution a far larger surface in proportion to its bulk than do the other forms. When washed clean from intestinal contents it dies in a ·6% solution of pure sodium chloride with a rapidity which makes experiment difficult. In neutralised laboratory saline it lives much longer, and still better in saline of which the reaction is faintly alkaline.

A. Chemotaxis.

After treatment for 10—15 minutes with an alkaline solution Opalina shows the most lively activity. Apparently the optimum reaction is one just on the alkaline side of neutrality. In a solution of such reaction Opalina becomes very transparent and moves with rapid rotation round its longitudinal as well as round a vertical axis, presenting an iridescent play of colour when viewed by reflected light. In stronger alkali, up to ·005% NaOH, it still moves vigorously, but the movements become more sluggish after treatment for some time, and in a few hours almost cease. In a faintly alkaline solution I have kept Opalina in active condition for 24 hours.

In alkaline solutions Opalina shows a marked tendency, both in the

watch-glass and under the coverslip, to form aggregations or clusters. In these the animals move with undiminished activity and speed, continually gliding over one another and rotating, but never going far beyond the outer limit of the cluster. A similar phenomenon, observed by Jennings[15] in his experiments on Paramœcium, was proved by him to be due to chemotactic attraction to carbonic acid. The other reactions of Opalina point to a similar cause in this case. The formation of such clusters naturally interferes with the exhibition of the normal chemotactic reactions and it was found necessary to disperse them, by tapping the coverslip, before the test-capillaries were introduced. With this precaution Opalina, after treatment for upwards of a quarter of an hour with any alkaline solution, was tested on the slide with test capillaries containing ·15 % acetic acid and ·1 % caustic soda, respectively, dissolved in ·6 % saline[1]. It showed an immediate and unmistakable attraction to the mouth of the acid capillary and an equally manifest repulsion from the alkali. A crowd of Opalinæ gathered round the mouth of the acid tube, and a widening clear space, which formed round the mouth of the alkaline tube, marked the progress of diffusion of the alkali. Five minutes after preparation it was usually found that all the Opalinæ in the drop had collected round the mouth of the acid tube, and examination with a low power of the microscope usually showed that some had already entered it. The intensity of the attraction varied with the alkalinity of the medium. If the alkalinity was marked 15 minutes, preliminary treatment was unnecessary for the exhibition of these phenomena: in fact the animals might be shaken directly into a drop of such solution (*e.g.* ·005 % NaOH in ·6 % NaCl), covered immediately and tested with the capillaries, and show an attraction to acid practically as immediate. When the solution was only faintly alkaline very inconsistent results were obtained if the preliminary treatment with the solution for some time was omitted. Such results, however, were often instructive. A common type of reaction, when the organisms were shaken directly into such a faintly alkaline or neutral solution on the slide, or left in it for only a few minutes in the watch-glass before testing, was a marked preliminary attraction to the alkali. This might last as long as 10 minutes, and showed itself by the formation of a very dense cluster at the mouth of

[1] The soda solution was first made up by adding 1 vol. of N soda to 3 of ·75 % neutral saline, 1 vol. of this being then diluted with 9 of ·6 % neutral saline. To this ·1 % NaOH the acetic acid solution was adjusted, so that a mixture of equal volumes was neutral to litmus.

the alkaline tube. After a variable time this cluster moved bodily away to the mouth of the acid tube, where a tendency to break up made its appearance, one Opalina after another leaving the cluster and entering the acid tube, which, after a time, became blocked at some distance from the mouth by a plug of Opalinæ. When the tube had become thus blocked a further reaction in several cases made its appearance; the individuals which were still collected round the mouth of the tube again formed a cluster and again moved away to the mouth of the alkaline tube, the contents of which were by this time (usually ½ an hour to an hour from the time of preparation) much weakened by diffusion. Here this somewhat loose cluster remained, attached to the side of the alkaline tube near its open end, but not moving actually opposite to the mouth and showing no tendency to break up and enter the tube. Fig. (3) is a copy of a diagram made from such a preparation

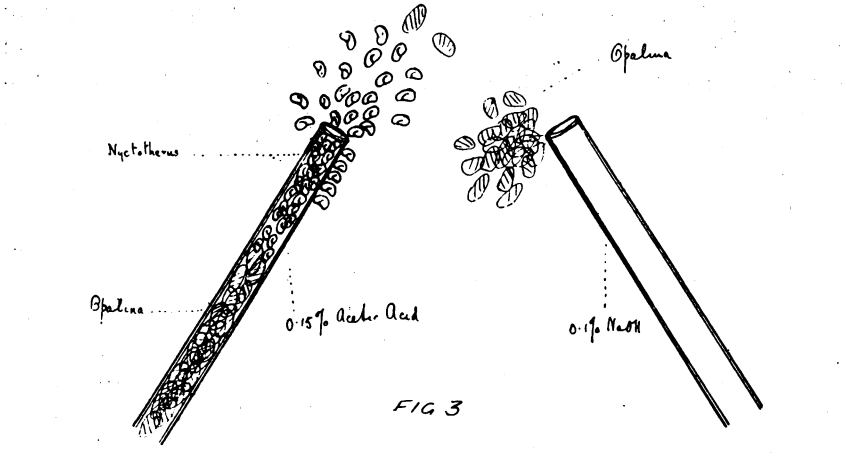


Fig. 3. A mixture of Opalina and Nyctotherus tested with tubes of acid and alkali. Weakly alkaline medium.

Opalina and Nyctotherus had been shaken into a weakly alkaline solution from a freshly-killed frog. A preliminary attraction to the alkali had been succeeded by collection at and entry of the acid tube, which became blocked by a plug of Opalina. This was followed by the entry of Nyctotherus into the upper part of the acid tube (*vide infra*); but meanwhile the rest of the Opalinæ had moved away and formed a cluster at the side of the alkaline tube as shown in the figure, which represents the state of affairs about 1 hour after preparation. Such a reaction may be said to be characteristic of a preparation of Opalina,

from a *freshly-killed* frog, in faintly alkaline solution. The meaning can, I think, hardly be mistaken. The rectal contents in the living animal have, almost always, a faint but distinct acid reaction, so that the Opalinæ are slightly acidified when taken from the rectum. If time is not given for this condition to be corrected by the action of the solution, the first reaction is a repulsion from acid and an attraction to alkali. When the condition of the animals has become altered by the action of the solution in which they are prepared, assisted by the alkali diffusing from the capillary near which they collect, a secondary and more marked repulsion from alkali and attraction to acid sets in, which may be followed, in the case of a few individuals which have become acidified at the mouth of the acid tube, by a third phase of repulsion from the acid and attraction to the alkali. It must be pointed out, however, that the alkaline effect is probably mitigated by the formation of clusters.

When the animals are obtained from a frog which has been dead for some hours, the attraction to acid is seen to be immediate and continuous; but under such circumstances the rectal contents are often so alkaline that a comparatively small quantity will reverse the reaction of a watch-glassful of ·005 % H_2SO_4. Under such conditions then it is to be expected that Opalina will react as after treatment with strong alkali; it will be seen that such is the case.

In neutral solution Opalina must be kept for some hours before experiment to ensure uniformity of result. After such treatment it shows an attraction to acid similar to that seen after treatment for some time with faintly alkaline solution.

Sodium carbonate may be substituted for sodium hydrate in the test solutions, and other organic acids, such as lactic or formic, for acetic acid without changing the general course of these reactions. If mineral acids are used the effects are again similar, but the attraction is weaker, or rather, is more easily replaced by secondary repulsion. If a preparation in neutral or faintly alkaline saline be tested with ·1 % H_2SO_4 and ·08 % NaOH, the first attraction to the acid tube gives way in a few seconds to a movement to the alkali, which may again be succeeded by movement back to the acid. Opalina, in fact, is extremely sensitive to the action of mineral acids and caustic alkalis. If very dilute test-solutions are used, such as ·005 % H_2SO_4 and ·004 % NaOH, a permanent attraction to the acid tube is seen, whether neutral or alkaline saline is the medium used for preparation.

It is very difficult to keep Opalina in a state of activity sufficient

for experiment in a solution made artificially acid, whether with organic or mineral acid. In such a medium, even when the acidity is slight, the movements become sluggish, the protoplasm opaque, and the outline of the animal more rounded. No tendency to form clusters is exhibited in this condition, the animals tending rather to adhere by their edges in the solution like inert bodies. It may be seen, however, that a general movement occurs from the neighbourhood of the acid to that of the alkaline tube, round which the accumulation is denser than elsewhere in the drop. Such a reaction, taken in conjunction with the behaviour of animals obtained from the fresh rectum, indicate that, at a certain stage of acidification, Opalina is repelled by acid and attracted by alkali. For the same reason a plug formed in the acid capillary, from an alkaline medium, was found after a time to move back to the mouth of the capillary and out into the drop, now become more strongly alkaline by the unequal diffusion resulting from the blocking of the acid tube.

It seems then that, in any condition of less than a certain weak acidification, Opalina will move towards acid and away from alkali; that in an acid solution its cilia rapidly become immobilised; but that at a stage still far short of complete immobilisation a tendency appears to move away from acid and towards alkali. In other words, when its condition is above a certain level of activity it moves towards acid, which tends to reduce its activity to that level; but when the activity passes below that level a tendency appears to move away from acid and towards alkali. The latter has a stimulating effect, and, having restored the activity to what I may call the *critical* condition, has a repellent action. The result must be that Opalina, in a medium of varying reaction, will collect in a region of faint acidity.

B. Galvanotaxis.

In observing this response, the same precautions with regard to preliminary treatment are necessary as in the case of chemotaxis. It is unnecessary to repeat the details, and it may be stated at once that, after suitable treatment with an alkaline or neutral solution, Opalina invariably collects at the anode. At closure of a strong current (9—12 cells), after a latent period of a few seconds, the animals set themselves with the anterior ends directed towards the anode, and swim to that side of the trough. With a weak current the orientation is less marked and permanent, the animals frequently

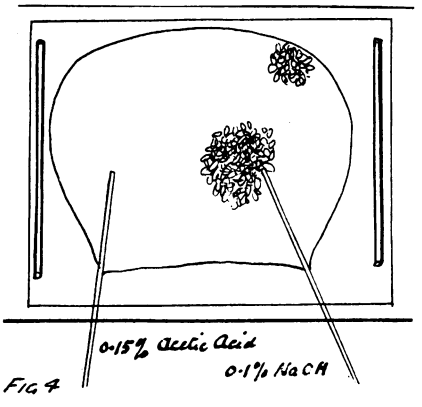
rotating, as is their normal habit, when not exposed to stimulation: but the prevailing disposition is with the anterior end towards the anode, motion in that direction taking place between each revolution, so that ultimately all collect on the anodic side of the cell. When the current is reversed, passage across to the opposite side, now the anode, begins after a few seconds and takes place in exactly the same way.

If the animals are shaken from the rectum of a freshly-killed frog into faintly alkaline or neutral saline and stimulated immediately, attraction to the *kathode* generally appears at closure. This may be as marked as the attraction to the anode seen after longer treatment with an alkaline solution. When a preparation which showed this kathodic galvanotaxis was tested with solutions it was found that the attraction to alkali was marked. After a time, varying with the intensity of reaction of the solution, the more usual attractions to acid and the anode appear. The following is the record of an experiment in which the comparison was carefully made.

Exp. Opalina obtained in great numbers from a freshly-killed frog, from the rectum of which it was shaken into laboratory saline coloured with litmus. Several drops of ·1 % H_2SO_4 added, but reaction is still distinctly alkaline. Specimen tested with current in stimulation trough.

3 cells—practically complete collection at kathode.

5 cells—more rapid collection at kathode.



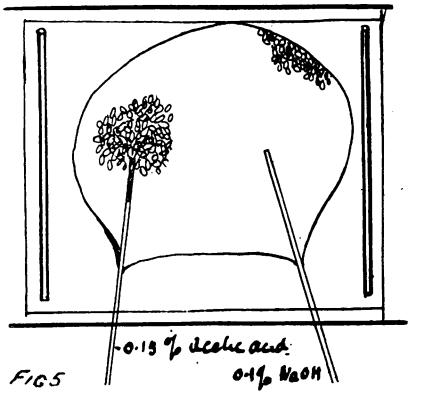


Fig. 4. Chemotaxis of Opalina from a freshly-killed frog, soon after shaking into very weakly alkaline saline.

Fig. 5. Chemotaxis of Opalina from a freshly-killed frog, after 15 minutes in very weakly alkaline saline.

8 cells—collection at kathode with tendency to swim transversely to lines of current.

10 cells—the same.

Slide preparation made immediately and tested with capillaries containing ·15 % acetic acid and ·1 % NaOH. Close cluster forms quickly at mouth of alkaline capillary (Fig. 4).

Other clusters formed in parts of the drop out of range of the test solutions[1]. After five minutes a tendency to move away from the alkali became apparent and the cluster gradually moved across bodily to the mouth of the acid tube. At the end of 15 minutes the condition represented in Fig. 5 had been reached.

At this stage another specimen was taken from the watch-glass and tested with the current (Fig. 6).

3 cells—anode

5 cells— „

8 cells— „

10 cells— „

The collection at the anode was now as marked with all strengths of current as the former collection at the kathode. At the end of a further five minutes the acid tube was found to be blocked by a plug of Opalinæ.

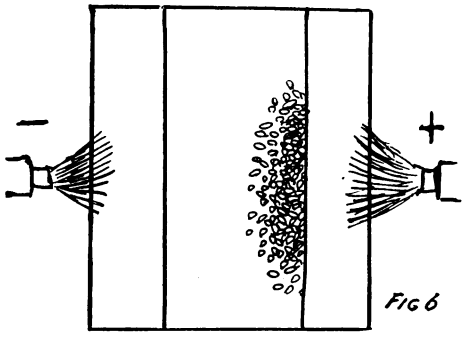


Fig. 6. Galvanotaxis of opalina in weakly alkaline medium.

Opalina artificially acidified likewise shows a tendency to collect at the kathode, but its sluggishness hinders the exhibition of this as it does that of the chemotactic reaction.

With regard to galvanotaxis also, we may speak, then, of a *critical* condition of Opalina. When in a condition of weaker acidification than this it is attracted to the anode; when in a condition of stronger acidification, to the kathode. Further, the critical condition for galvanotaxis corresponds as far as can be ascertained with that for chemotaxis. With so sensitive a form as Opalina it is impossible to observe a chemotactic reaction such as would correspond to this condition—viz. repulsion from both acid and alkali, since the application of the test-solutions is quite sufficient in itself to alter the condition. With the current it can sometimes be observed. If the

[1] It would appear that the acidified Opalinæ are still attracted by carbonic acid, though repelled by stronger acids.

organisms are shaken from a fresh frog into a moderately alkaline solution (*e.g.* laboratory saline) in the trough, and tested at frequent intervals, there may often be found a condition in which the first kathodic attraction can no longer be detected while the attraction to the anode has not yet appeared. In this condition a weak current appears to be without effect, while a strong current causes swimming *transversely* to its direction. If we may suppose that the latter reaction is expressive of repulsion from both poles the phenomena are those we should expect to be exhibited by animals in the critical condition.

If such a preparation be left for some minutes and again tested it is found that the ordinary anodic galvanotaxis has now appeared.

The response of Opalina to the two kinds of stimuli may be summarized as follows for purposes of comparison.

	Alkalinated	Neutralised	Acidified
Chemotaxis	Attraction to acid Repulsion from alkali	Attraction to acid Repulsion from alkali	Attraction to alkali Repulsion from acid
Galvanotaxis	Collects at anode	Collects at anode	Collects at kathode

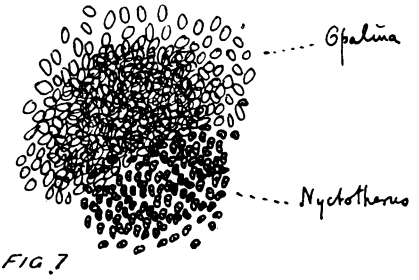
Nyctotherus cordiformis.

This species is very much more resistant to the action of solutions than is Opalina. It can be kept for several hours without apparent injury in tap-water, and for several days in normal saline. The time necessary for a given solution so to alter its condition as to change its reactions to test-solutions, or the current, is correspondingly greater. A solution of very weak reaction will usually produce its effect on Opalina, so as to secure uniformity of response, in 15 minutes or less; but I have kept Nyctotherus in such a solution for 48 hours without obtaining absolute uniformity in the reaction of individuals. No reaction is recorded here, however, in which a practical uniformity of response was not obtained. To secure this it was found that treatment with solutions of marked reaction—*e.g.* ·005 % NaOH or H_2SO_4—for 30 min. sufficed; with neutral solutions and those of weaker reaction treatment was often continued for 24 hours.

A. Chemotaxis.

As in the case of Opalina, some hint of the chemotactic reactions of Nyctotherus may be obtained simply from observation of its behaviour in a watch-glass filled with salt solution. When the solution

is alkaline it shows a tendency to aggregation somewhat similar to that seen in the case of Opalina. This tendency is immediately manifest when the animals are taken from a frog some hours dead, appearing only some time after preparation when they are taken from a freshly-killed frog. When, as is usually the case, Opalina and Nyctotherus are examined together they form, in a solution of marked alkalinity, a combined cluster at the bottom of the glass. If the solution is of weaker reaction a somewhat remarkable behaviour is to be observed. The two species at first form a combined cluster; but Nyctotherus soon begins to leave this and to form a cluster of its own, so that after a time two adjacent clusters are formed each consisting entirely of one species (Fig. 7).



Clusters of Opalina and Nyctotherus in a faintly alkaline medium.

The cluster of Nyctotherus is of a rather different nature from that of Opalina, the animals being disposed in a single layer. This is probably connected with the tendency of Nyctotherus to swim close to any surface with which it comes into contact, its flattened side being closely applied to such a surface—*e.g.* the bottom of the watch-glass.

If we are right in supposing that the clustering of Opalina is due to attraction to acid, and may further suppose that the broad-surfaced Opalina envolves CO_2 more rapidly than the compact Nyctotherus, this behaviour might be taken as indicating that Nyctotherus shows a weaker attraction to acid, when in weakly alkaline solution, than does Opalina—a suggestion which is supported by the experiments with test-solutions.

When strongly alkalinated—as when prepared from a stale frog or treated for some hours with laboratory saline—Nyctotherus shows an attraction to the acid which is not conspicuously different from that of Opalina.

When treated only for a shorter time with such a solution, or for a long time with a very weak one, after preparation from a fresh frog, it shows an attraction to the acid which is conspicuously inferior to that of Opalina. Under such conditions it often shows a preliminary

attraction to the alkaline tube long after that of Opalina has disappeared and does not collect near the mouth of the acid tube until that has become blocked by a plug of Opalina. In this way it is common to have two collections formed in the acid tube at different levels, the lower one consisting entirely of Opalina, the one nearer the mouth entirely of Nyctotherus (see Fig. 5)—a phenomenon which strikingly recalls the formation of separate clusters in the watch-glass. It must be borne in mind that, under such conditions as those represented in Fig. 5, the diffusion from the acid capillary is greatly hindered, while that from the alkaline capillary continues unimpaired. It is probable, therefore, that the reaction of the outside solution is alkaline nearly up to the mouth of the acid capillary, and that repulsion from alkali probably plays more part in the behaviour of Nyctotherus than attraction to acid. Experiments made with preparations containing Nyctotherus *only*, which had been treated for 6—24 hours with a solution only just alkaline, showed that it was attracted, in this condition by very weak acid, but repelled by stronger acid and by alkali of any strength. It showed, in fact, a tendency to collect near the acid tube when this was first inserted, followed by a movement away to a part of the solution distant from both tubes, but nearer to the acid.

The resistance of Nyctotherus to solutions, while it necessitates longer treatment to produce a given condition, makes that condition more permanent when produced. Nyctotherus, for example, shows, in the alkalinated condition, a more permanent attraction to weak mineral acid than does Opalina, in which contact with such an acid rapidly produces a change of condition.

It is evident from the facts mentioned above that the attraction of Nyctotherus to acid declines very rapidly as the alkalinity becomes weaker. After treatment for 24 hours with a neutral solution it shows repulsion from acid and alkali which are practically equal. The response varied a little in either

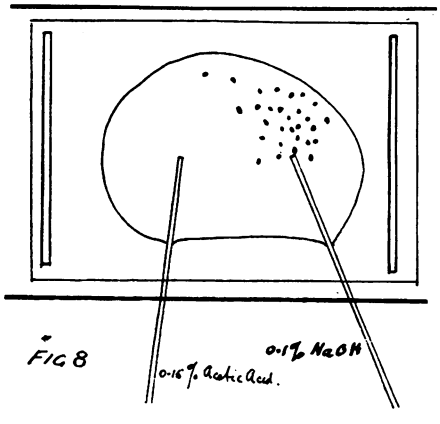


Fig. 8. Chemotaxis of Nyctotherus after several hours' treatment with acid saline.

direction in various experiments, and in one case no difference could be detected in the effects of the two test-solutions, Nyctotherus moving away from both without apparent inclination to the side of either.

After treatment for a suitable time—24 hours was allowed with solutions of weak reaction—with an acid solution Nyctotherus shows distinct attraction to the alkaline capillary.

Fig. 8 shows the response after 24 hours' treatment with a solution made very faintly acid with acetic acid.

In an acid solution Nyctotherus shows no tendency to aggregation, becoming widely distributed through the solution.

B. Galvanotaxis.

After such treatment with alkaline solution as results in attraction of Nyctotherus to acid it collects at the anode with all strengths of current used in these experiments. The orientation in lines of current is not marked unless the animals are strongly alkalinated. When taken from a stale frog, or treated with markedly alkaline solution for half-an-hour it collects at the anode with a rapidity not perceptibly different from that of Opalina. When shaken from a freshly-killed frog into moderately alkaline solution and tested after a few minutes, it is seen that Opalina collects with much greater rapidity at the anode than does Nyctotherus, which remains scattered evenly through the solution when Opalina has formed a complete collection. If the current is allowed to run for some minutes longer Nyctotherus also collects, as a rule, at the anode. In many cases, however, after such short treatment Nyctotherus collects at the kathode, while Opalina collects at the anode. Longer treatment always produces the anode-seeking tendency in Nyctotherus also.

The following experiment illustrates this point, showing that Nyctotherus may retain the kathode-seeking tendency for a perceptible time even in a markedly alkaline solution.

EXP. Opalina and Nyctotherus shaken directly from a freshly-killed frog into ·6 % saline, with ·01 % NaOH, in the stimulation trough. Tested after an interval of about one minute with

Current from 3 cells—Opalina collects at anode. Nyctotherus at kathode.

" " 6 " " " " " " " "

" " 9 " " " " " " " "

Left for 10 minutes and again tested.

Current from 3 cells—Opalina and Nyctotherus both to anode.
6 cells— „ „ „ „ „
9 cells— „ „ „ „ „

Here, then, is probably the explanation of Verworn's observation that Opalina collects at the anode, Nyctotherus at the kathode. His result was probably due to this unequal sensitiveness of the two forms to solutions, Opalina being affected in a few minutes by a solution which may take hours to affect Nyctotherus.

In weakly alkaline solutions (as also in neutral or weakly acid) Nyctotherus shows to strong currents (9—12 cells) a reaction of a somewhat peculiar nature. The animal sets itself with its longer (antero-posterior) axis *across* the lines of current and with the side on which the mouth opens turned towards the kathode. It is further to be seen that, in the majority of cases, the anterior end of the animal is turned to the *right* of the direction in which the current flows—*i.e.* to the right hand of an observer looking in the direction of the positive stream. When the current is reversed all quickly rotate, turning towards the aboral side, so as to take up the same disposition with regard to the new direction of current.

A few individuals can be seen which have the anterior end directed to the left of the positive stream. This behaviour, which for some time seemed inexplicable, I believe to be due to the disposition of parts in the animal. One side of the body is flattened and is applied to any surface with which the animal comes into contact—in this case to the floor of the stimulation chamber. Now it can be observed that the relation of parts is such that, when the mouth is directed to any point, the anterior end is directed, in the majority of individuals, towards the right hand of an observer looking at that point, in a minority of cases to his left hand. The result is that, when, at the make of a strong current, each animal turns with its mouth towards the kathodic side of the chamber, the majority show a tendency to swim to the right of the positive stream, the minority to the left. In alkaline solution there was at the same time a tendency to swim backwards to the anode, the resulting course being diagonal and leading ultimately to the formation of unequal collection in what may be called the right and left anodic corners of the chamber (see Addendum, p. 360).

After long treatment (24 hours) with carefully neutralised solution no trace of reaction to weak currents (3—6 cells) could be detected. With the stronger currents (9—12 cells) there appeared the curious

orientation across the lines of current described above. This, however, was in this case attended by only a slow translatory motion, although the animals, when unconstrained by the current, still moved freely in the solution. In most cases a very slow movement to the anode was perceptible with the strongest current used, but only so much as caused the appearance of a narrow free space on the kathodic side of the chamber after passage of the current for some time. In one preparation not even this trace of attraction to the anode could be detected after 10 minutes' passage of a current from 12 cells; and this preparation was the one above referred to as having shown, with acid and alkali, an apparently equal repulsion from both. The following are the details of another experiment:

EXP. Nyctotherus left all night in neutralised solution giving a rather bluish purple colour with litmus.

Chemotaxis. There is a tendency to form a loose cluster in the solution. Testing with ·15 % acetic acid and ·1% NaOH shows—

(1) Brief preliminary movement towards acid tube.

(2) Movement away from both tubes (Fig. 9).

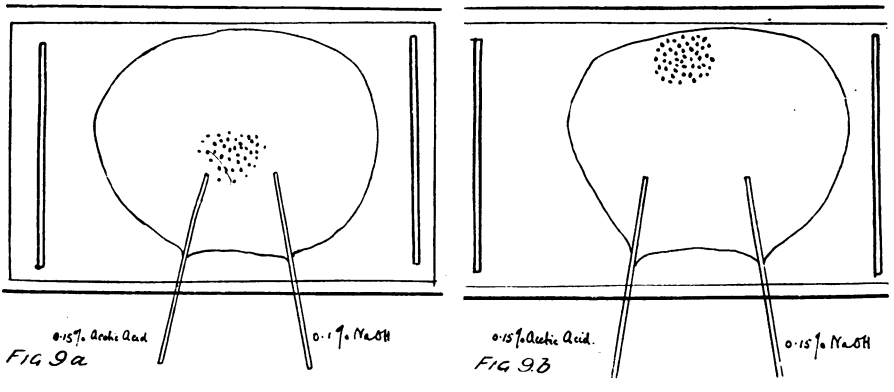


Fig. 9. Chemotaxis of Nyctotherus after 24 hours' treatment with neutral saline. (*a*) 1 min. (*b*) 10 mins. after preparation.

Galvanotaxis. 3 cells—no effect.

6 „ „

9 „ „ except transverse setting.

12 „ (current passed for 10 minutes). Narrow clear space appears on kathodic side of the chamber (Fig. 10).

In other cases a slight preliminary attraction to alkali corresponded to a weak attraction to the kathode appearing only with 12 cells. After treatment with an acid solution Nyctotherus always collects at the kathode. If the solution is of marked acid reaction and the treatment sufficiently long it sets with the anterior end towards the kathode and swims straight to that pole in the direction of the current. When acidified to a smaller extent, as by a few hours' treatment with a very weakly acid solution, it shows the transverse orientation with strong currents which it exhibited in neutral and weakly alkaline solutions, but now moves diagonally to the *kathodic* corners of the trough. As mentioned above, the kathode-seeking habit is characteristic of Nyctotherus taken from a freshly-killed frog. When shaken from the fresh rectum into alkaline saline it may sometimes be seen, by testing at intervals with the current, to pass through the various stages of response corresponding to conditions varying from marked acidification to marked alkalination, viz.:

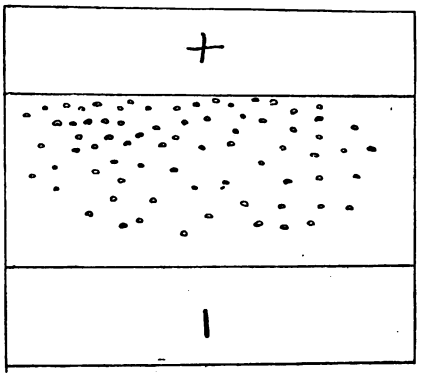


FIG 10

Galvanotaxis of Nyctotherus after 24 hours' treatment with neutral saline (giving bluish and purple reaction with litmus). 12 cells.

(1) Movement to kathode directly and with some longitudinal orientation.

(2) Movement to kathode with transverse orientation.

(3) Transverse orientation only.

(4) „ „ with movement to the anode.

(5) Movement to the anode, with some longitudinal orientation.

It is necessary at this point to discuss the possible cause of this curious transverse orientation with the mouth towards the kathode. The suggestion is obvious that it expresses a repellent effect of both anode and kathode, the repulsion being stronger in the one case or the other when movement to kathode or anode takes place, and equal in the two cases when motion of translation is absent or takes place only in a completely transverse direction. If this is the case we have a very interesting correspondence between repulsion from acids and alkalis (*vide* results of chemotaxis) and repulsion from anode and kathode.

This still leaves unexplained, however, the invariable turning of the mouth towards the kathode. The only hint of an explanation of this reaction was given by an experiment in which I happened to test Nyctotherus in a weakly alkaline solution, not with the usual weak test-solutions in the capillaries, but with capillaries containing 1 % H_2SO_4 and ·8 % NaOH respectively. It could then be seen that when, after the preliminary attraction to acid, the animals felt the effect of the alkaline solution they turned with their mouths towards the alkaline capillary and moved diagonally away from both tubes (Fig. 11).

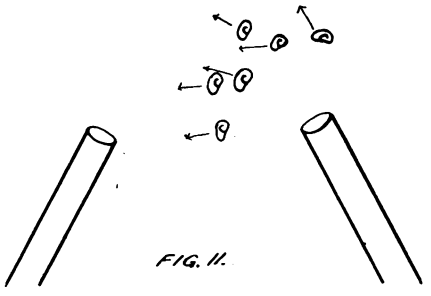


FIG. 11.

It seems, then, that Nyctotherus turns its mouth towards the stronger alkaline test-solutions as it turns it towards the kathode of a strong current. This, of course, leaves the meaning of both phenomena quite unexplained, but it furnishes another instance of the remarkable parallelism between the chemotactic reactions of this species to acids and alkalis and its galvanotaxis when tested under similar conditions. The reactions of the two kinds may be summarized as follows for purposes of comparison and with the omission of all purely theoretical deductions.

	Strongly alkalinated	Weakly alkalinated	Neutral	Weakly acidified	Strongly acidified
Chemotaxis.	Attraction to acids.	Attraction to very weak acids. Repulsion from stronger acids & alkalis of all strengths.	Repulsion from both acid and alkali, apparently equal with equivalent solutions.	Attraction to weak alkalis. Repulsion from stronger alkalis and acids of any strength.	Attraction to alkali. Repulsion from acid.
Galvanotaxis.	Attraction to anode.	Attract. to anode, with transverse setting to strong currents.	Transverse setting with slow transverse motion. No movement to either pole.	Attraction to kathode with transverse setting to strong currents.	Attraction to kathode.

Balantidium entozoon.

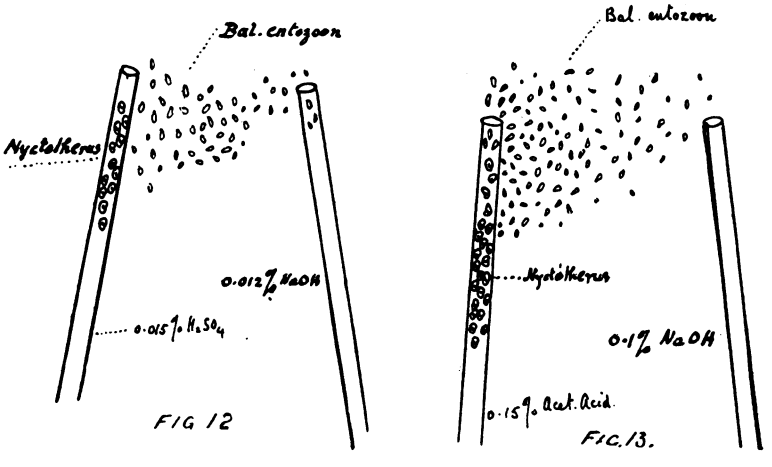
This, the smallest Balantidium found in the frog, occurs together with Opalina and Nyctotherus at the junction of the small intestine with the rectum. It shows a very conspicuous tendency to collect round shreds of fæcal matter, and adheres to these with such tenacity

that it is very difficult to obtain a preparation of the animals in clean salt solution, free from such particles.

A. Chemotaxis.

This tendency to adhere to solid particles in the solution interferes very considerably with the exhibition of chemotactic reactions by this species, and I have not hitherto been able to obtain results as definite in this case as in that of Nyctotherus. The main features of its behaviour, however, have been definitely made out, partly by the slide and cover-glass method used for Opalina and Nyctotherus, partly by the use of capillaries in the watch-glass.

In alkaline solution, after treatment for not less than an hour, it collected round the mouth of a capillary containing ·15 % acetic acid, or entered the capillary, according to its condition. Such a reaction indicates no difference between this form and Nyctotherus or Opalina. More instructive results can be obtained by examining it in a preparation which also contains Nyctotherus. Under such circumstances it can be clearly seen that the attraction of Balantidium to acid changes to repulsion at a lower point of concentration of the acid than does that of Nyctotherus. This is especially the case if mineral acid be used. Fig. 12 is taken from a preparation of Nyctotherus and B. entozoon, treated for many hours with very weak alkali, and tested with ·015 % H_2SO_4 and ·012 % NaOH. It represents the condition an hour after insertion of the capillaries.



Figs. 12 & 13. Chemotaxis of Nyctotherus and Balantidium entozoon. Weakly alkaline medium.

Fig. 13 is from another specimen of the same preparation tested with the usual acetic acid and soda solutions.

It will be seen that Nyctotherus has entered the acid tube and formed a collection at some distance down from the mouth, the Balantidia assembling in an area near the end of the tube on the side towards the alkaline capillary.

When taken from a frog some hours dead, or when treated with a markedly alkaline solution, the attraction to the acid becomes more marked and causes passage into the tube of ·15 % acetic acid.

With stronger test-solutions a diphasic reaction was in many cases obtained. With a 1 % solution of acetic acid and a roughly equivalent solution of sodium carbonate this species in alkaline solution showed a tendency to aggregate near the acid tube which appeared very quickly and lasted for several minutes. Then, while Opalina and Nyctotherus remained near the acid tube, and entered it after some time, Balantidium moved away, collected at the mouth of the tube of sodium carbonate, and eventually entered this.

I have not yet been able to make certain of the reactions of this species when neutralized. Left for a few hours in a neutral solution it shows variations of behaviour corresponding, presumably, to differences in its condition at the commencement of the experiment. Left for longer periods in neutralised saline it dies and disintegrates. It can only be said, then, that after several hours in an apparently neutral solution it sometimes shows a weak attraction to acid, appearing long after the insertion of the capillaries, when the test-solutions must have become much weakened, sometimes a somewhat earlier weak attraction to alkali.

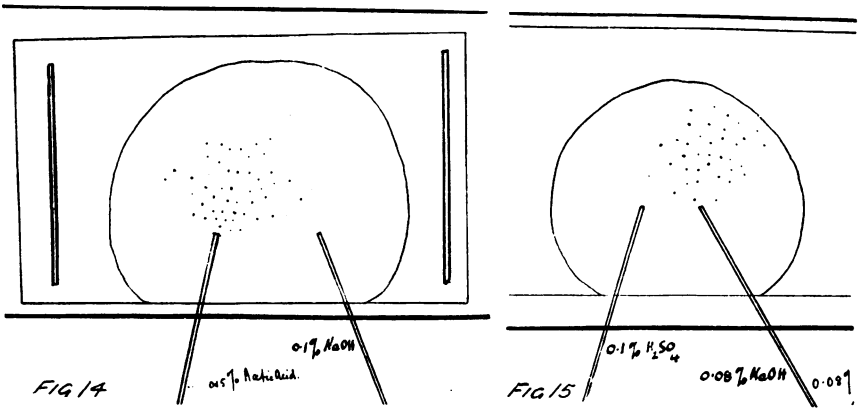
Fig. 14 is taken from a preparation in which Balantidium was treated for 6 hours in a solution giving a rather bluish-purple reaction to litmus. It represents the distribution an hour after insertion of the solutions.

If under such conditions a mineral acid—*e.g.* 0·1 % H_2SO_4—is substituted for the acetic no such attraction to the acid can be detected, the distribution inclining rather to the alkaline side of the drop (Fig. 15).

In acid solution, after treatment for some hours, Balantidium entozoon shows a marked attraction to alkali.

No critical condition, such as that described in Nyctotherus, has as yet been produced in Balantidium entozoon. It may be doubted, indeed, whether such exists, but further discussion of the matter may be

postponed till the reactions of the other species of Balantidium have been described, since these have many points in common.



Figs. 14 & 15. Chemotaxis of Balantidium entozoon. Weakly alkaline medium.

B. Galvanotaxis.

With weak currents the galvanotaxis of Balantidium entozoon resembles that of Nyctotherus, collection occurring at the anode in alkaline solution, at the kathode in acid solution, while the reaction after long treatment with neutral solution is very slight. With stronger currents collection at the kathode always occurs.

These points are illustrated by the following experiments.

Exp. Opalina, Nyctotherus and Balantidium entozoon shaken into saline with ·01 % NaOH and left for half-an-hour. Tested in trough with

Current from 4 cells—all collect at anode.
10 cells—Opalina and Nyctotherus to anode.
Balantidium to kathode.

Exp. Opalina, Nyctotherus and Balantidium entozoon shaken into saline which was neutralised and left for one hour, at the end of which time the litmus in solution was still of a neutral purple colour. Tested with

3 cells—Opalina—to anode.
Nyctotherus—no reaction.
Balantidium—„ „
6 cells—Opalina—to anode.
Nyctotherus—no reaction.
Balantidium—small collection forms at kathode.
12 cells—Opalina—rapidly to anode.
Nyctotherus—very slowly to anode.
Balantidium—completely to kathode.

EXP. Opalina, Nyctotherus and Balantidium entozoon shaken from a frog some hours dead (rectal contents markedly alkaline) into laboratory saline. Tested with current from

3 cells—all collect at anode.
6 cells—„ „ „ „
Current increased to 9 cells—Opalina and Nyctotherus remain at anode. Balantidium entozoon moves off to kathode.
12 cells—Opalina and Nyctotherus to anode. Balantidium to kathode.

The following is the record of an experiment made on the galvanotaxis of a specimen from the preparation of which the chemotactic reaction is represented in Figs. 16 and 17.

EXP. Bal. entozoon treated with neutral solution giving a bluish-purple with litmus for one hour.

3 cells—no perceptible effect.
6 „ „ „ „
9 „ to kathode.
12 „ „ „

It should further be pointed out that, in the experiments recorded above, a weak current was passed for some time and its strength suddenly increased by the opening of a short-circuiting key. If through a markedly alkaline preparation a strong current was passed, without previous passage of a weaker current, its first effect was to cause collection at the anode. If it was allowed to run for a few minutes it could be seen that the attraction to the anode passed off, and the Balantidia, one by one, left the anodic side of the chamber and, setting themselves in the lines of current with the anterior end directed to the kathode, swam straight to that pole, where in a short time, they all collected. If the current was reversed all now passed straight across to the new kathode.

This effect recalls inevitably the diphasic reaction observed with the stronger test-solutions and a strongly alkalinated preparation.

It is clear that the reactions of Balantidium entozoon to the current correspond roughly with its chemotactic reactions, particularly if mineral acids are used. The parallellism is not so complete as that which could be made out in the case of Nyctotherus; but on the other hand the experiments are less complete, and owing to the peculiarities of the species are necessarily of a rougher nature. It is hoped that a more satisfactory method may be discovered of applying the test-solutions.

For the present the results may be summarized as follows:

	Alkalinated	Neutral	Acidified
Chemotaxis.	With weak solutions—attraction to acid. With stronger solutions—attraction to alkali (diphasic effect).	With organic acid (?) to acid after long diffusion. With mineral acid (?) to alkali.	To alkali
Galvanotaxis.	With weak currents—attraction to anode. With stronger current—diphasic reaction. Preliminary attraction to the anode, succeeded after a few minutes by attraction to the kathode.	With weak currents—(?) With strong current—to kathode.	To kathode

Balantidium elongatum.

This large form, from the lower end of the duodenum, is not nearly so regular an inhabitant of the intestine of the English frog as the three species already dealt with.

Since the breeding season I have found a few frogs which yielded a fair supply and have been able to confirm and amplify the somewhat rough experiments made in the winter, when the supply was abundant.

A. Chemotaxis.

When first shaken from the intestine of a freshly-killed frog, in which the zone inhabited by this species appears to have a neutral or faintly alkaline reaction, Balantidium elongatum appears very sluggish. The body is rather sharply curved and the animal swims in curves, turning towards the concavity of the body-curvature (Fig. 16).

FIG. 16.

A few minutes' stay in an alkaline solution suffices to modify greatly the appearance of the animal. The body becomes straightened out and, at the same time, longer and thinner, and the animal now swims in approximately straight lines, making sharp angles in its course. It also shows a marked tendency, particularly if the solution is strongly alkaline, to rise to the surface and swim in the surface film. When the alkalinity is reduced so that the reaction approaches the neutral point this tendency disappears and a close collection is formed at the bottom of the watch-glass. For this behaviour I can, at present, suggest no explanation. When a slide preparation of the Balantidium is made it appears in the form of a marked tendency to swim in the surface film at the edge of the drop, leaving the centre practically empty unless the

number of animals in the drop is very large. This naturally interferes with the method of testing on the slide usually adopted for other forms. It may, however, be used as follows.

The drop is prepared under the cover-glass in the usual way. If it is kept under observation, it will be seen that the Balantidia, which, when the drop is covered, swims almost immediately to the edge, show, after some minutes, a tendency to return to the middle. This tendency increases, and in a few minutes they become evenly distributed through the whole drop. If the capillaries are now inserted attraction to one or the other may be observed. After a time, however, the attraction to the peripheral film again appears and is often sufficient to neutralise the attraction of a test solution.

A much more satisfactory way of applying the test-solutions in the case of this species is to push the ends of the capillaries into a collection of the animals at the bottom of a watch-glass. Even when the tendency

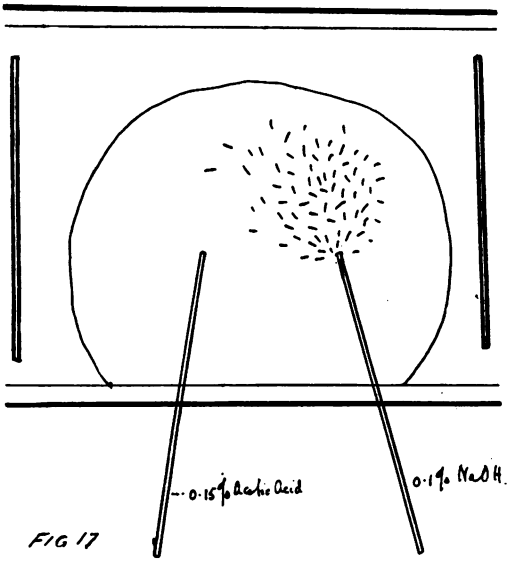


Fig. 17. Chemotaxis of Balantidium elongatum. (Slide and cover-glass method.) Medium alkaline.

Fig. 18. Chemotaxis of Balantidium elongatum. (Tested in a watch-glass.) Medium weakly alkaline.

to swim in the surface-film is most marked, such an aggregation may be obtained by a gentle rotatory motion of the watch-glass.

By both these methods it was found that alkali exerted a distinct

attraction for this species, when treated with acid, neutral, or even moderately alkaline solution for a few hours. Even after several hours in laboratory saline they swam right up into the alkaline tube from the watch-glass. The acid tube, after ½ an hour, did not contain a single individual. Figs. 17 and 18 represent the effect of test solutions on B. elongatum, treated for ½ an hour with laboratory saline, showing respectively the reaction on the slide and in the watch-glass.

Only after prolonged treatment with moderately alkaline solution (*e.g.* 24 hours in laboratory saline), or after an hour or two in very markedly alkaline solution is this attraction to alkali replaced by attraction to acid. Even then the attraction to acid is a weak one, a collection forming near the mouth of a capillary containing ·15 % acetic acid when the slide-method is used, and a few individuals swimming a short way into the acid tube when the test is applied in the watch-glass (Fig. 19).

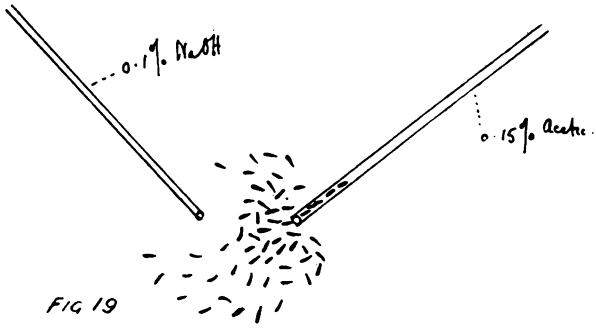


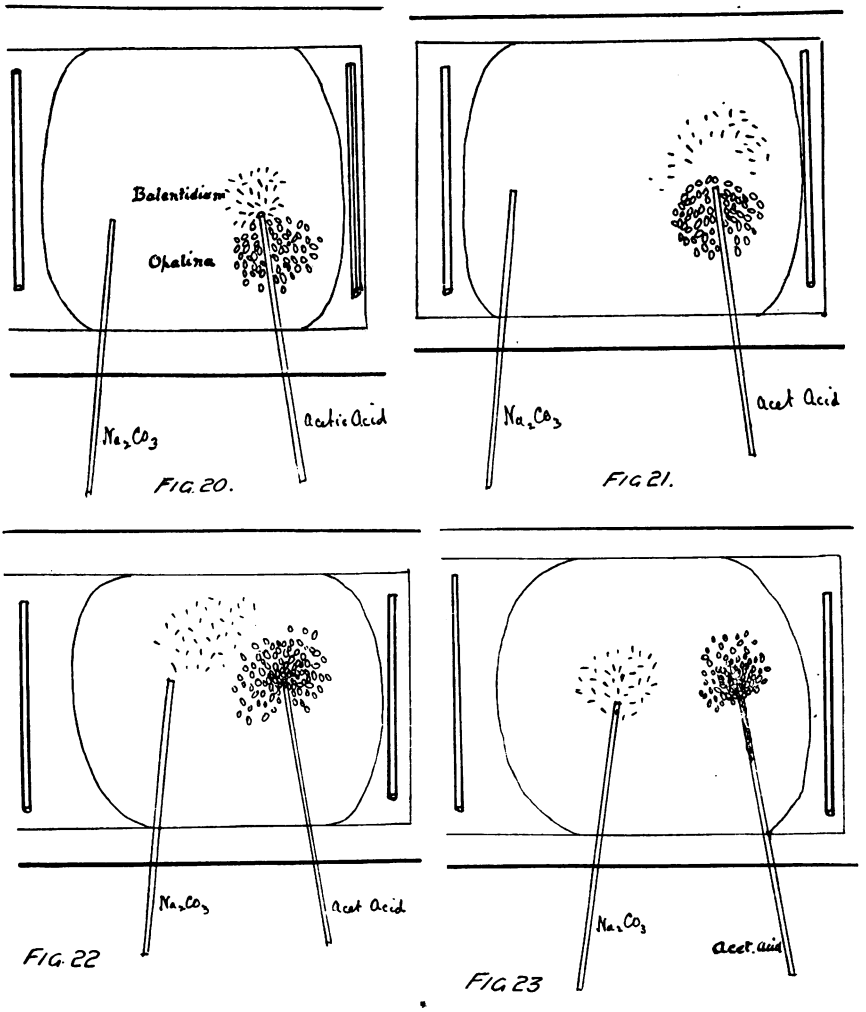
Fig. 19. Chemotaxis of Balantidium elongatum. (Tested in a watch-glass.) After prolonged treatment with markedly alkaline saline.

When B. elongatum is abundant it may be found, in a frog some hours dead, among the now strongly alkaline contents of the rectum, mingled with Opalina and the other species normally found in that region.

Specimens prepared under such conditions show attraction to the acid tube with weak test-solutions. When stronger solutions were used—*e.g.* 1 % acetic acid and equivalent sodium carbonate—a very marked diphasic reaction was obtained.

Figs. 20, 21, 22 and 23 show four stages of an experiment made with such a mixture of Opalina and B. elongatum, shaken from the rectum of a stale frog into laboratory saline.

In this experiment the Balantidium, being a more rapid swimmer, collects at the mouth of the acid tube more quickly than Opalina. By



Figs. 20—23. Chemotaxis of a mixture of Opalina and Balantidium elongatum from a stale frog. Alkaline medium.

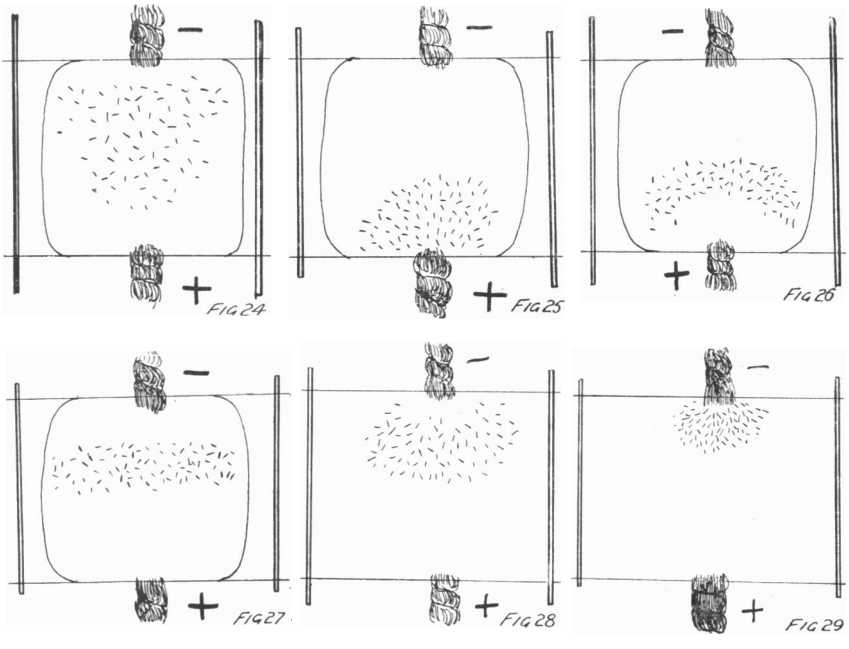
Fig. 20. 1 minute after preparation.
Fig. 21. 5 minutes „ „
Fig. 22. 10 mins. after preparation.
Fig. 23. 20 mins. „ „

the time that the attraction of Opalina has become marked (5 mins. after preparation) the secondary repulsion of Balantidium has already begun.

B. elongatum differs, then, in its chemotactic behaviour from the forms previously described, in that it shows, under all ordinary circumstances, a tendency to collect in alkali. Only after strong alkalination does it form aggregations in weak acid, and, even under such conditions, passes over to the alkali when both acid and alkali become stronger by diffusion.

B. Galvanotaxis.

The characteristic tendency of B. elongatum is to collect at the kathode. In neutral, acid, or moderately alkaline solution (except after long treatment), a few seconds after make of a current through the solution it turns its anterior end towards the kathode, and swims straight to that end of the chamber. The orientation is more complete and permanent with strong currents when the solution is neutral or acid. In the latter case the appearance of a body curvature, similar to



Figs. 24—29. Galvanotaxis of Balantidium elongatum from a stale frog. Alkaline medium.

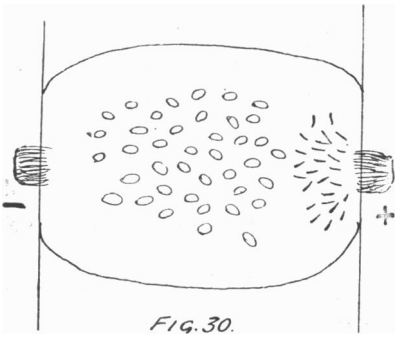
Fig. 24. Before closure.	Fig. 27. 15 mins. after closure.
Fig. 25. 5 mins. after closure.	Fig. 28. 30 mins. ,, ,,
Fig. 26. 10 mins. ,, ,,	Fig. 29. 35 mins. ,, ,,

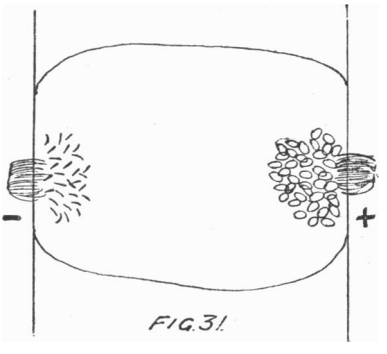
that seen in the animal as shaken from a fresh frog, hinders the direct passage to the kathode. In alkaline solution there appears, with strong currents, a tendency to swim across the current to the sides of the trough, so that collections form in the kathodic corners.

After treatment with strong alkali (*e.g.* ·015 % NaOH) for an hour, or for 24 hours with laboratory saline, collections are formed at the anode with weak currents: but the anodic becomes replaced by a kathodic tendency if the weak current is run for a long enough time, or immediately if its strength is increased.

The diagrams in Figs. 24—29 are taken from an early experiment made by the rough method of leading the current from the non-polarisable electrodes through cotton ropes soaked in saline to the solution containing the animals under a cover-glass; but the effect of very long passage of a weak current (exact strength unknown, but E. M. F. probably about equivalent to that of 4 bichromates—*i.e.* from 6—8 volts) is so well shown that the record seems worth reproduction. The animals were shaken from the small intestine of a stale frog into laboratory saline.

In Figs. 30 and 31 are represented two stages of an experiment made in the same way with a mixture of Opalina and Balantidium elongatum from the rectum of a stale frog. The resemblance to the





Figs. 30 and 31. Galvanotaxis of Opalina and Balantidium elongatum from a stale frog.

Fig. 30. 5 minutes after closure of a weak current.
Fig. 31. 20 „ „ „ „

first and last stages of the experiment represented in Figs. 21—24 is striking and suggestive.

The following are specimen records of more recent experiments with the stimulation-trough.

EXP. Balantidium elongatum shaken from lower end of duodenum of a freshly-killed frog into laboratory saline. Neutralised roughly and left for an hour. Divided into three portions.

(1) Made markedly alkaline by addition of half its volume of ·04 % NaOH in ·6 % saline.

(2) More carefully neutralised.

(3) Made distinctly acid by addition of a little ·1 % H_2SO_4.

After about an hour tested with current.

(1) *Alkaline solution.*

3 cells—doubtful. Some to anode, others unaffected.

6 „ „ „ „ „ „

9 „ —kathode } with swimming across lines of current.

12 „ „ }

(2) *Neutral solution.*

Passage to kathode direct and immediate with all strengths.

(3) *Acid solution.*

Passage to kathode complete with all strengths, but hampered by revolving of animal towards concavity of curve of body.

Preparation (1) left all night and tested again next day.

3 cells—all go to anode.

6 „ —most go to anode: a few to kathode.

9 „ — to kathode { but without complete orientation, animals occasionally turning and swimming back short way to anode.

12 „ „ „ { All ultimately collect at kathode.

EXP. Balantidium elongatum from junction of small intestine and duodenum shaken into laboratory saline. Resulting solution distinctly alkaline to litmus.

3 cells—after two minutes' passage, incomplete collection at kathodic end.

6 „ —more complete collection after two minutes (Fig. 33).

9 „ —further increase of kathodic tendency.

12 „ —yet further increase of kathodic tendency. Reversal causes immediate passage to new kathode.

EXP. Opalina, Nyctotherus and Bal. elongatum from rectum of stale frog shaken into laboratory saline. Part neutralised, and to rest added ·01 % NaOH to increase alkalinity. Left for five hours and then tested with current in stimulation-trough.

(1) *In neutral saline.* Collected at bottom of watch-glass, where litmus is reddish.

3 cells—Opalina—anode.
Nyctotherus—anode (slowly).
Balantidium—no reaction.

6 cells—Opalina—anode.
Nyctotherus— ?
Balantidium—kathode.

12 cells—Opalina—anode.
Nyctotherus—across current to right.
? slight tendency to kathode.
Balantidium—kathode.

(2) *In alkaline saline.*

3 cells—Opalina, Nyctotherus and Balantidium—anode.

6 cells—Opalina and Nyctotherus—anode.
Balantidium—kathode.

Solution poured off and replaced by saline with ·01 % NaOH. Left for 10 minutes. Then—

6 cells—Opalina, Nyctotherus and Balantidium—anode.

12 cells—Opalina and Nyctotherus—anode.
Balantidium—kathode.

The following experiment gives an opportunity of comparing the reaction to the current of Balantidium elongatum with that of Opalina, Nyctotherus and Balantidium entozoon.

EXP. Contents of rectum of a stale frog—Opalina, Nyctotherus, Balantidium entozoon and elongatum—shaken into salt solution containing ·0025 % acetic acid.

Resulting solution gave a red colour with litmus, which changed to purple in an hour or two and still gave a purple reaction on the next day. Reaction may be called neutral, therefore. Tested with weak current.

Result—Opalina, Nyctotherus and B. entozoon collect at anode.

Bal. elongatum collects at *kathode.*

It is obvious that the kathode- and alkali-seeking condition is much more easily produced in Balantidium elongatum than in the three other species. It is again doubtful whether a "critical condition" can be spoken of, since strong alkalination does not seem to weaken the attraction to strong alkali or to the kathode of a strong current; it merely introduces an anodic and acid attraction with weaker currents and solutions. The reaction may be tabulated as follows:

	Strongly alkalinated	Weakly alkalinated	Neutralised	Acidified
Chemotaxis.	*Weak solutions* — attraction to acids. *Strong solutions* — attraction to alkali. (Diphasic action.)	Attraction to alkali.	Attraction to alkali.	Attraction to alkali.
Galvanotaxis.	*Weak current*—attraction to anode, giving way to attraction to kathode with very long passage. *Strong current* — short attraction to anode, quickly giving way to kathodic attraction.	Attraction to kathode.	Attraction to kathode.	Attraction to kathode.

Balantidium duodeni.

This species, which in the autumn I found in many frogs in such extraordinary abundance that the duodenum had a mottled white appearance, disappeared in the spring, and I have since found it only on two occasions in sufficient abundance to make satisfactory experiment possible. The early rough experiments gave somewhat inconsistent results, presumably due to differences in reaction of the duodenum at various parts of its length, and insufficient care in giving long treatment with solutions of known reaction before making the experiment. The correspondence, however, of chemotactic with galvanotactic phenomena was always decided when experiments of the two kinds were tried on the same specimen. For this reason the results obtained already with this species seem worth recording. It is hoped that the examination of its reactions may be completed a few months hence.

Chemotaxis and Galvanotaxis.

Two special features in the behaviour of this species make the examination of its reaction and its treatment with solutions for any length of time peculiarly difficult.

In the first place it exhibits so strong a thigmotactic adhesion to the bottom of the watch-glass into which it is shaken that the fluid may be poured off or drawn into a pipette without loosening the hold. If the animals are loosened with a camel's hair brush or a shred of blotting-paper, stirred into the solution and then drawn into the pipette, they adhere to the inside of the pipette unless the fluid is blown out very quickly. This tendency involves the loss of a large proportion of individuals at every transference from one solution to another, and necessitates a large supply of material if successful experiments are

to be made. The solution of this difficulty will probably be found in the application of both chemotactic and galvanotactic tests in the watch-glass.

In the second place it shows a very marked tendency to swim to the extreme edge of the solution and even beyond, dragging a thin film of salt-solution with it. Here it stays until killed by dehydration, due to the concentration of the film of salt-solution by evaporation. This tendency is probably due to the attraction exerted on this species by strong solutions of NaCl. If the end of a capillary containing 5 % NaCl be introduced into a group of these animals in the watch-glass, the mouth soon becomes surrounded with a swarm and ultimately plugged by a mass of the creatures. It must, however, also be mentioned that this attraction to the edge of the solution is seen, to a very marked degree, only in such specimens as show attraction to alkalis; that it is soon corrected by the action of a markedly alkaline solution; and that it is not seen in a markedly acid solution. It seems therefore to be connected with attraction to alkali as well as to sodium chloride. The reactions after treatment with markedly acid and alkaline solutions have therefore been studied with success, the species exhibiting marked attraction to alkali and the kathode in the former, to acid and the anode in the latter case. It is in studying the reaction after treatment with weaker and neutral solutions that the difficulty becomes serious and has, hitherto, prevented satisfactory observations. Experiments made by shaking the animals direct from a freshly-killed frog's duodenum into such solutions on the slide, or in the stimulation trough, usually indicated the existence of a diphasic reaction to both kinds of stimuli—attraction to weak acid or the anode of a weak current, and to stronger alkali or the kathode of a stronger current. Experiments in which the animal was left for a few hours in such solution also indicated that it was peculiarly resistant to their action—a specimen which showed attraction to the kathode and to alkali when first shaken into slightly alkaline saline exhibiting the same reactions after an hour's treatment, while faintly acid saline produced no greater effect on a specimen showing a previously developed attraction to acid and to the anode. Stronger solutions act on it, apparently, with a rapidity out of proportion to the greater percentage of free acid and alkali. The following records of experiments with two different specimens illustrate this point.

EXP. Balantidium duodeni from duodenum of a freshly-killed frog shaken into laboratory saline. Resulting reaction distinctly alkaline. Left for an hour and tested with current of eight cells.

Result—distinct collection at anode (Fig. 32).

Salt solution made faintly acid with H_2SO_4 and left for one hour. Tested with eight cells.

Result—collection still at anode.

Sblution made markedly acid by addition of about $\frac{1}{10}$ of its volume of ·1 % H_2SO_4. Left for 10 minutes.

Tested with eight cells.

Result—rapid collection at the kathode.

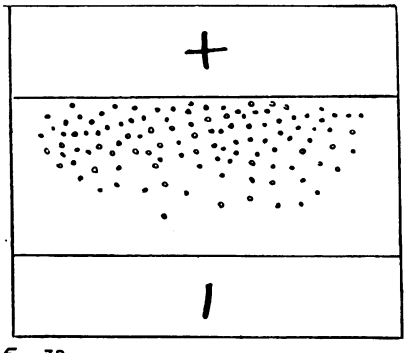


FIG 32

Fig. 32. Galvanotaxis of Balantidium duodeni. Moderately alkaline medium.

Such an experiment by itself might indicate that there was a critical reaction for Balantidium duodeni at a certain stage of acidification. There are experiments however in quite the opposite direction, as the following:—

Exp. Balantidium duodeni shaken into laboratory saline. Resulting reaction weakly alkaline. Tested with current of 3, 5, 8 and 10 cells.

Result—collection at *kathode* in every case.

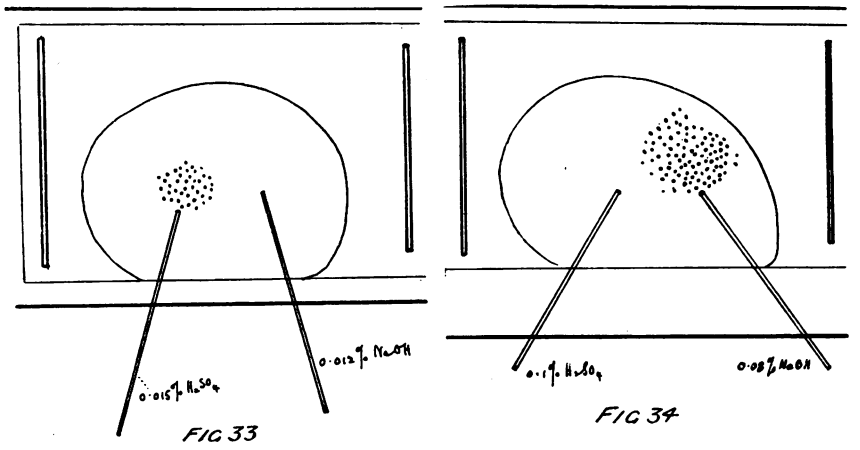
Left for four hours and again tested with a result indistinguishable from the previous one.

This specimen tested in the watch-glass with capillaries containing ·1 % H_2SO_4 and ·8 % NaOH. Considerable number enter alkaline tube; none in the acid, even after leaving for several hours.

The diphasic reaction to solutions is seen in the experiment represented in Figs. 33 and 34, in which B. duodeni shaken from the duodenum of a stale frog into neutral saline was tested with solutions.

These represent, respectively, the reaction to solution containing ·015 % H_2SO_4 and ·012 % NaOH, and ·1 % H_2SO_4 and ·08 % NaOH.

It will be seen that in the former case attraction to the acid occurs, in the latter to the alkali.



Figs. 33 and 34. Chemotaxis of Balantidium duodeni from a stale frog. Neutral medium. Weak test-solution.

In a species so resistant to the action of reagents it seems doubtful whether this attraction to stronger alkali can be explained by immersion in weak acid, such as is brought about during the preliminary attraction to acid, which appeared in the experiment represented in Fig. 36 before the final collection at the alkaline tube. It appears, rather, as if there were indeed attraction in the case of weaker solutions to the acid, in the case of stronger to the alkali under the same conditions—viz. when the animal is examined in neutral solution. Such an idea is borne out by the observation, for a long time puzzling, that, with a moderate current, addition of weak acid to the solution, into which the animals are directly shaken and tested *at once*, increases the attraction to the anode, while addition of stronger acid causes a reversal of effect, attraction to the kathode appearing. This result may be due simply to the different initial reactions of the successive specimens used, but its regular appearance whenever the experiment was made seems to point to another origin. I hope to follow the question further as soon as an opportunity of obtaining Balantidium duodeni in quantity presents itself. The following is a typical experiment of this kind.

Exp. Balantidium duodeni, from a stale frog, shaken directly, from successive small pieces of duodenal wall, into solutions of increasing acidity in the stimulation trough and tested with a current from six cells immediately.

Results. (1) Neutral solution—no effect.
(2) ·005 % HCl—collects at anode in about five minutes.
(3) ·01 % HCl—collects at anode in about five minutes.
(4) ·015 % HCl—collects at *anode* in about three minutes.
(5) ·02 % HCl—collects closely at *kathode* in about two minutes.

Reversal of current was not tried in this case, since the essential feature of the experiment is the application of the test before the solution has time to affect the condition of the animals. Results of a very similar nature were obtained when alkaline solutions of increasing strength were used, the change from anodic to kathodic attraction taking place in this case when the strength of alkali passed a certain point. The effect produced by the solution as, so to speak, an addition to the external stimulus can be distinguished from its effect, after longer action, on the animal's condition, as in the following :—

EXP. Bal. duodeni shaken directly into ·016 % NaOH in the trough and current made immediately. Collects rapidly at the kathode. Current allowed to run. After some minutes movement away from the kathode sets in and collections are now formed in the anodic corners.

The diphasic reaction in nearly neutral solution is exampled in the following :—

EXP. Balantidium duodeni shaken into pure NaCl ·6 % and tested with varying strengths of current.
3 cells—anode.
6 cells—anode.
12 cells—anodic collection which passes off in two minutes and is replaced by kathodic. Reversal of current—pass over at once to new *kathode.*

It may be provisionally suggested that the immediate effect of acid or alkali added to the solution in these cases is to intensify the effect of anode or kathode, causing in the one case anodic repulsion, in the other kathodic attraction to appear at a lower strength of current than when the experiment is made in a solution of weak reaction. This would be expected if the view is adopted that the diphasic reaction really indicates attraction to weak acid or the anode of a weak current, and to stronger alkali or the kathode of a stronger current.

If this is so we are probably justified in supposing that the diphasic reactions of the other species of Balantidium have a similar origin—a supposition which would tell strongly in favour of the theory that the chemotaxis to acids and alkalis and galvanotaxis in salt solutions are closely related phenomena, a point to which we may return later.

More careful experiments on Balantidium duodeni are necessary, however, before decision on this point is possible, and for the present it must be sufficient to have pointed out the general and, in most cases, striking parallelism between the two classes of reaction as exhibited by different species under varying conditions.

The question as to the meaning of the difference in reaction between the various species remains untouched. It may be doubted, for example, whether the prevalence of the acid-seeking condition in Opalina, as compared with Balantidium elongatum, means more than that the Balantidium is much more resistant than is Opalina to the action of alkaline media. The important point for our present purpose is that the difficulty of producing the *acid-seeking* state is closely paralleled by difficulty in producing the *anode-seeking* state, and that an individual which exhibits the one reaction always, as far as can be seen, exhibits the other. Theoretical considerations as to the possibility of explaining the one class of phenomena in terms of the other may be postponed until experimental results of another kind have been described.

IV. Ciliary action under the influence of chemical and electrical stimuli.

The changes produced in the ciliary action of Paramœcium and other forms by mechanical and chemical stimuli have been described by Jennings[8a]. He describes a reversed stroke of the cilia of Paramœcium on contact of its anterior end with a foreign body or a repellent chemical stimulus, followed by reversed striking on the aboral side only, the result being that the animal swims back from the stimulus and then rotates towards the aboral side, and swims away in a new direction. This is termed a "motor reflex," and reactions of a similar type are described in other species.

The changes produced by the current in the motion of the cilia have been described by Ludloff[3] in Paramœcium, and by Pearl[8] in various other species. The general result obtained is that, after a more or less prominent "motor reflex," or attempt to turn from the kathode, the animals are orientated in the lines of current by the action of the cilia, which strike forwards on the kathodic, backwards on the anodic side (distinguished, by Pearl, from the "motor reflex" as "forced movement").

The animal then, with its cilia striking in the directions represented

diagrammatically in Fig. 35, moves to the kathode, for reasons which are not too clear.

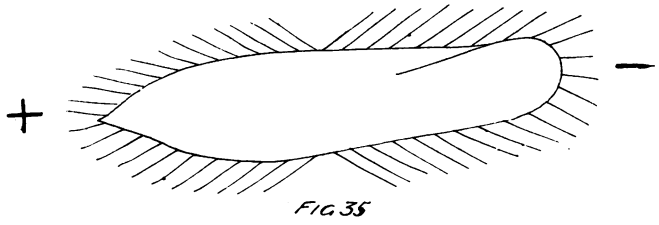


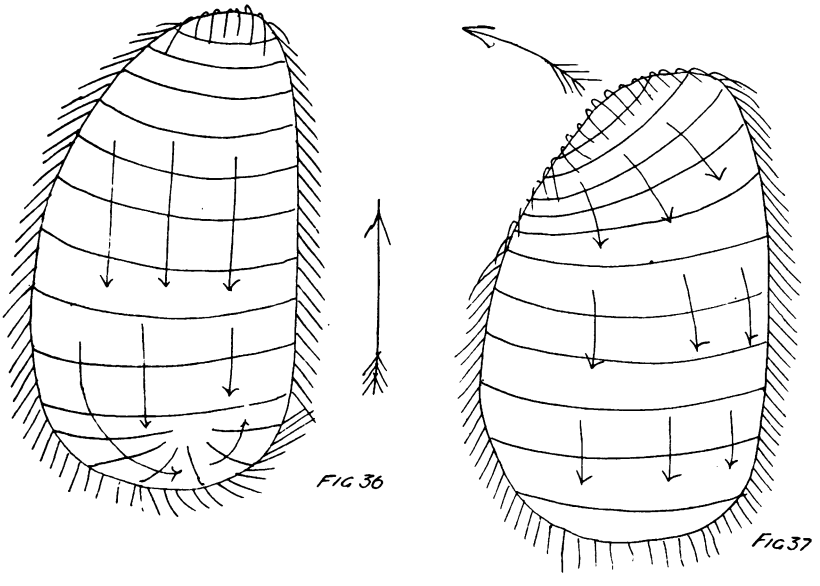
Fig. 35. Diagram of ciliary action of Paramœcium under influence of the current. (After Ludloff.)

It is suggested by Ludloff that the backward-striking cilia on the anodic side are more effective because the cilia are striking in the normal direction. In a few repetitions of these experiments I was able to see the effects described by these observers.

All experiments hitherto having been made with species inhabiting water and tested in that medium, it seemed worth while to examine the ciliary action of species tested in salt-solutions. Observations were made on Opalina and, with somewhat less success, on Nyctotherus.

For stimulation with the current, Opalina and Nyctotherus were prepared in a weak solution of gelatine, resembling that used by other observers (Pearl, Jennings, Ludloff, etc.), except in that the solution was made in ·6 % saline instead of in water. Three grammes of gelatine dissolved in 100 c.c. of laboratory saline was found to give a medium of the required consistency. A weaker solution did not sufficiently restrain the movements for observation with high objectives, while in a stronger solution the delicate Opalina was liable to become fixed in a wrinkled and contorted position from which it could not extricate itself. The jelly was warmed very gently in a watch-glass until it *just* melted; a drop of solution containing as large a number as possible of the required species was mixed with it; and a drop was then placed on a slide provided with electrode-holders, and covered with a slip before it had cooled. The current was led under the coverslip by applying the brushes of the electrodes directly to its opposite edges, or by applying them to slips of blotting-paper soaked in the gelatine solution and in contact with the solution under the coverslip. A current of 10—12 cells was generally used. In a few cases a weaker current (6 cells) was used.

Opalina. Normal ciliary action. If Opalina, swimming freely in neutral or weakly alkaline saline, is observed under a moderate power of the microscope (*e.g.* Zeiss C), it can be seen that the ciliary motion takes the form of a series of regular waves passing over the surface of the animal. This is described by Saville-Kent[14]. Under a high power ($\frac{1}{6}$ or $\frac{1}{8}$ obj.) it can be seen that the waves pass in the same



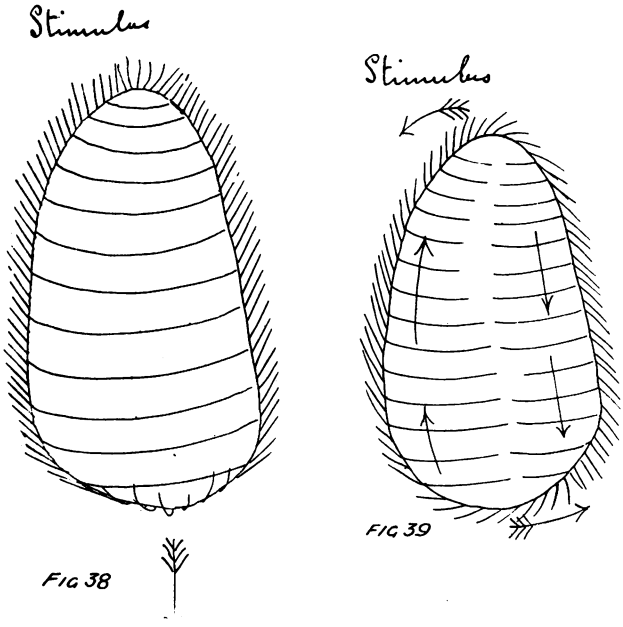
Figs. 36 and 37. Opalina. Two types of normal ciliary action.

direction as the effective beat of the cilia. It can also be seen that in some individuals, which for the time being are swimming straight forwards, the waves pass directly backwards from the anterior to the posterior end of the animal (Fig. 36); in others, which show the more characteristic rotatory motion, the waves pass obliquely across and backwards, from *the more* curved to the *less curved* lateral edge (Fig. 37).

The waves pass in the direction of the plain arrows, motion of the animal taking place in the direction of the feathered arrows in the figures.

Effect of chemical stimuli. If the animal, as it swims, comes into contact with an obstacle, or a repellent stimulus such as a solution of alkali, it responds with a motor reflex similar to that described by Jennings[8a] in Paramœcium and other forms. There is a momentary

reversal of all the cilia, which strike for about ½ a second towards the anterior end and drive the animal backwards, and it then turns quickly in the normal direction of rotation—*i.e.* towards the more curved edge—the turning being accelerated by continuance of the forward stroke by cilia on the more curved side, those on the straighter side resuming the normal backward stroke (Figs. 38 and 39). As it rotates, the cilia along



Figs. 38 and 39. Effect of a stimulus on the ciliary action of Opalina.

the curved edge cease to strike forwards, oblique waves, starting from the curved edge, reappear, and either continue to cause rotation, or come to start from points nearer and nearer to the anterior end until they start actually from that end and pass directly backwards (Fig. 36), so that the animal swims away from the stimulus.

On the other hand, when the anterior end of an Opalina, in alkaline solution, comes into contact with weak acid diffusing from a capillary, the oblique waves show a tendency to pass more directly backwards and bring the animal nearer to the point from which the diffusion starts. Contact of the lateral edge with acid seems to cause acceleration of the oblique waves causing rotation, until the anterior end is in the acid, when the waves pass directly backwards.

Effect of the current. If an Opalina is observed which is revolving with oblique waves of cilia before the current is made, it can be seen that at make of current there is no very obvious reaction, except in the case of a specimen which happens to be pointing towards the kathode, in which case the action resembles that seen on reversal of the current (*vide infra*).

From any other position the animal continues revolving and passes towards the anode-pointing position. As it approaches this the waves begin to pass more and more backwards, until they pass directly backwards when it reaches the anode-pointing position, so that it moves straight forwards towards the anode (Fig. 40).

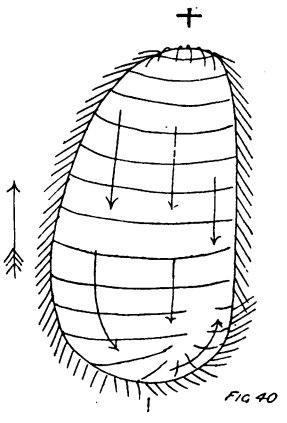


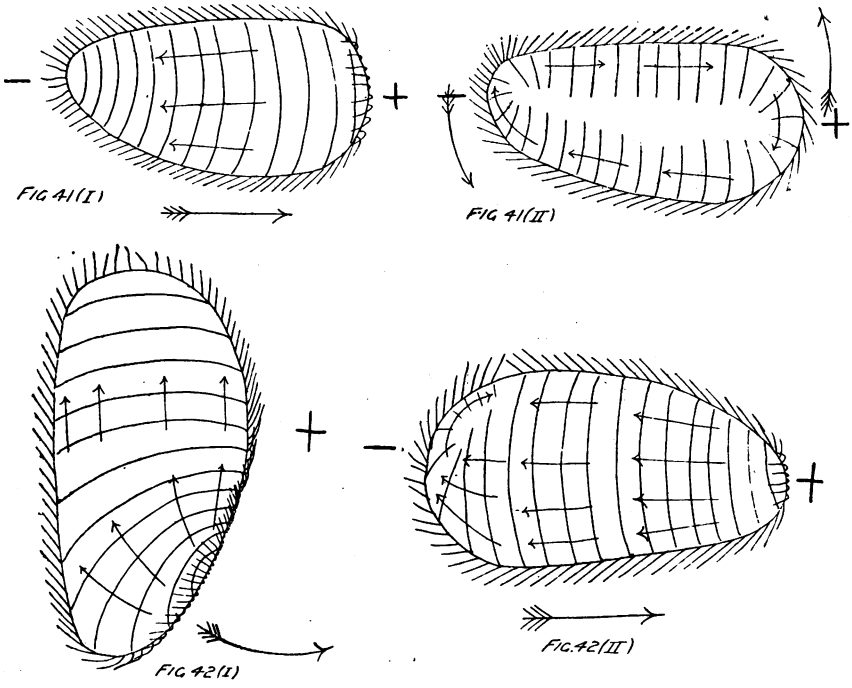
Fig. 40. Ciliary action of Opalina during anodic galvanotaxis.

The intermediate stages can be more satisfactorily studied by observing the behaviour at reversal of the current.

If, while the animal is in the anode-pointing position, the current is reversed the first effect has all the appearance of a stimulus to the anterior end, viz. a momentary reversal of the ciliary action driving the animal backwards from the new kathode (Fig. 41, I.) Then, while the cilia on the more curved side continue to strike forwards, those on the side of lesser curvature resume their backward stroke (Fig. 41, II.). The animal is by this action rapidly rotated into a position transverse to the current. As it comes into this position the direction of the ciliary waves changes so that they now start from a point on the more curved edge, nearer the anterior than the posterior end, and pass obliquely forwards and backwards (Fig. 42, I.) This action necessarily causes continued rotation in the same direction, and, as before, as the animal approaches the anode-pointing position the point of origin of the ciliary waves shifts to the anterior end, so that, when the animal has come into line with the lines of current, the waves again pass directly backwards (Fig. 42, II.).

It will be noticed that there is (Figs. 42, II. and 40) a small part of the kathodic end of the straighter edge where, in the anode-pointing position, the cilia strike forwards. This probably neutralises a slight tendency to rotation caused by very slight obliquity of the main ciliary waves. It is clear from the above description that rotation of the

anterior end to the anode at closure of current *always* takes place in the normal direction of rotation of the unstimulated animal. If, while a weak current is passing, the anterior end is carried, by spontaneous rotation, past the anode-pointing position, the animal

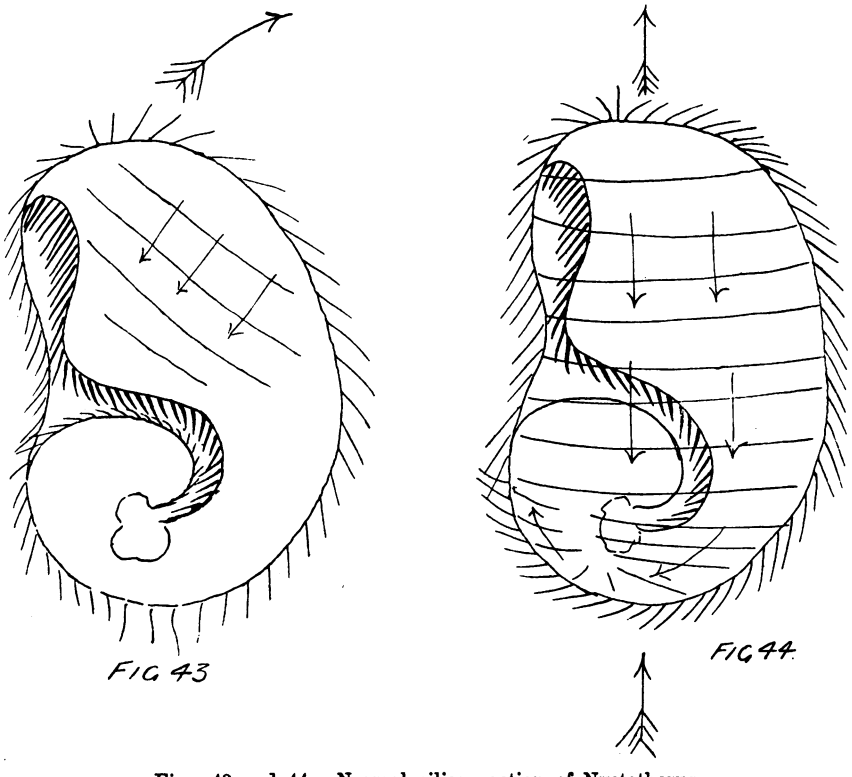


Ciliary action of Opalina. (Anodic galvanotaxis.) Changes at reversal of current.

returns to that position, not by rotating back towards the less curved edge, but by completing the revolution, in the course of which it goes through all the stages of ciliary motion corresponding to its positions, including a brief movement directly backwards as it comes into the kathode-pointing position.

Nyctotherus. The study of the ciliary motion of Nyctotherus is slightly complicated by the fact that the ciliation is not, as in the case of Opalina, uniform. The animal, indeed, is ciliated over its whole surface, but the cilia appear to be most strongly developed round the edge of the flattened body. Besides this general ciliation there is a row of very large and powerful cilia, the adoral cilia, developed along the edge of the peristome field and continued, as the pharyngeal cilia, to the bottom of the pharynx.

Normal ciliary action. As the animal swims ordinarily the stroke of all the cilia appears to be towards the hinder end. This fact is obscured when examination is made with a low power by the fact that the waves of motion of the adoral cilia pass in the opposite direction to the effective stroke, viz. towards the anterior end, and even appear to be continuous round the anterior end with waves passing along the cilia of the convex surface, which may conveniently be called the *dorsal* cilia. Examination with a high power reveals the fact that the effective stroke is always to the posterior end (Fig. 43).



Figs. 43 and 44. Normal ciliary action of Nyctotherus.

When the animal is moving, free from any constraining influence, in an alkaline solution, it rotates constantly towards the dorsal surface. This rotation is brought about, apparently, by the comparatively weak action of the dorsal cilia and by the addition of the effect of the powerful adoral cilia to that of the ventral ciliation. Also, somewhat

irregular waves may be seen passing obliquely across the lateral surface from the dorsal to the ventral edge. The motion as a whole is irregular, the rotation giving way at intervals to motion straight forwards, in which circumstances waves of ciliary motion can be seen to originate at the anterior end and pass straight backwards over the whole animal (Fig. 44).

Under such conditions a tendency to strike forwards is shown by the cilia behind the pharynx on the ventral edge (Fig. 44), which assist in neutralising the rotatory tendency produced by the stroke of the adoral cilia.

Another type of movement, referred to before, is that in which the body is kept in a certain fixed direction by balanced movement of ventral and dorsal cilia, while the general body ciliation propels the animal in the direction towards which the dorsal, or the ventral surface is directed (Fig. 13).

Effects of chemical stimuli. These were less definite than in the case of Opalina. Nyctotherus, in alkaline solution, aggregated at the mouth of a tube from which weak acid was diffusing still exhibited the general tendency to rotation towards the dorsal surface. Individuals which had left such a collection could, however, be seen to return to it by movement straight forwards with the second type of ciliary motion described above. The third type, in which motion of transference is at right angles to the longest diameter, has already been described as produced by the repulsion of the animal from the stronger alkaline test-solutions.

Effects of the current. The movements of Nyctotherus are obviously more complex than those of Opalina, and there is a corresponding variation and complexity in its reaction to the current.

Before stimulation with the current Nyctotherus was kept for 48 hours in a very weakly alkaline salt-solution. Under such conditions about 90 % showed unmistakable attraction to the anode. Of these some moved directly towards the anode, being orientated in the lines of current; others set themselves across the current, moving transversely and in most cases to the right, with the mouth turned towards the kathode, moving at the same time towards the anode, to which the dorsal surface was directed; others again moved very slowly to the kathode, with transverse motion, and occasionally rotated into the anode-pointing position, and moved, for a space, straight towards the anode.

The remaining few mostly moved straight, with orientation in lines

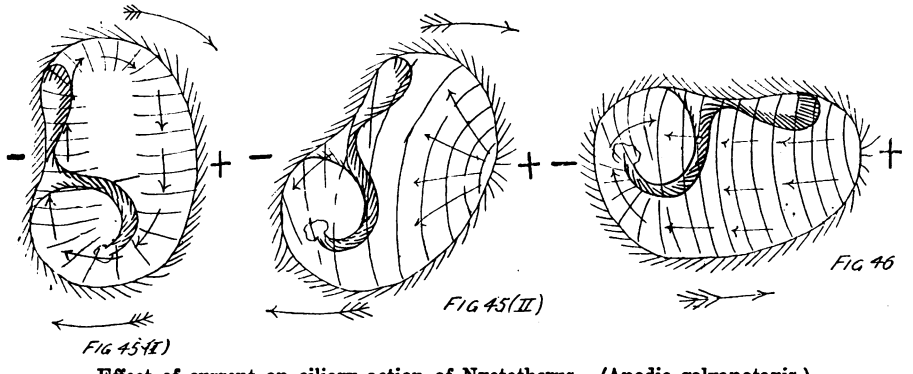
of current, to the kathode. Such a preparation, then, gave an opportunity of examining the ciliary action causing all the varieties of response described in this species. All except a small proportion collected ultimately at some point of the anodic side of the coverslip, except such as, reaching the lateral edge too soon, were caught there in the gelatine concentrated by evaporation.

These various types of movement can be reduced to two types of ciliary action, as follows :

(1) *Motion to either pole with orientation in lines of current.* At make of current the dorsal cilia, which were striking weakly in a backward direction, strike powerfully forwards and the animal rotates rapidly in an aboral direction (Fig. 45, I.). As it approaches the required position the dorsal cilia begin striking backwards again and their stroke gets stronger, while waves begin to pass, from a point near the anterior end, over the general ciliation (Fig. 45, II.), and, when the animal has reached the position of orientation, pass directly backwards so that it moves forwards towards anode or kathode, as the case may be (Fig. 46).

The post-pharyngeal ventral cilia may usually be seen to strike forwards in this position.

At reversal of current the dorsal cilia again strike forwards and rotate the animal into the opposite position. Rotation thus always takes place in the normal direction of rotation of the unstimulated animal.



Effect of current on ciliary action of Nyctotherus. (Anodic galvanotaxis.)

(2) *Transverse orientation.* This differs from the above reaction only in that the forward stroke of the dorsal cilia ceases and becomes replaced by a backward stroke as the animal comes into position

transverse to the current. The rotation is again always in the aboral direction and always brings the animal into the position in which its mouth is directed to the kathode. As stated above, in the majority of cases this means that the anterior end of the animal is directed to the right of the positive stream, in a minority to the left.

There is, apparently, no regular wave movement of the general body cilia to be seen under these conditions: as a rule, after the treatment described the general tendency was to strike effectively towards the mouth and drive the animal slowly towards the anode, while the marginal and adoral cilia drove it to the right (or left) of the current (Fig. 47). In a few cases the tendency was to strike towards the dorsal margin and drive the animal slowly towards the kathode; in such cases there was usually a periodic reversal of the dorsal cilia, bringing the animal round with the anterior end towards the anode,

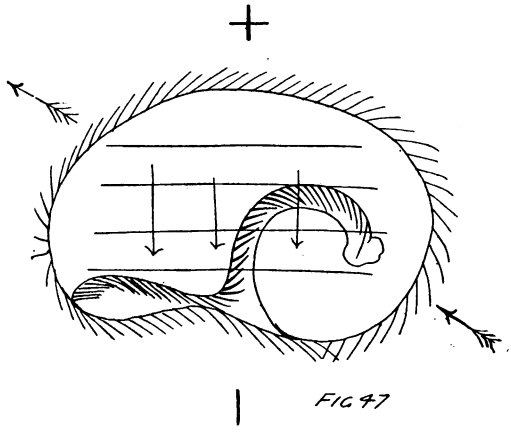


Fig. 47. Effect of current on ciliary action of Nyctotherus. (Transverse orientation.)

towards which it moved for a time with ciliary motion of type (1), and then, by another reversal of the dorsal cilia, completing the aboral revolution, resumed the transverse and slow kathodic movement, ultimately arriving at the lateral edge of the coverslip somewhere on the anodic half of that edge. At reversal of current a transversely set individual rotated into the opposite transverse position by exactly the same process of reversal of dorsal cilia and aboral rotation.

One other phenomenon only needs mention. A Nyctotherus which had travelled directly to the anodic edge of the coverslip, arriving

there and entering the zone where the solution was becoming concentrated by evaporation, frequently turned round and swam for a short distance directly towards the kathode, and, if the current was reversed while it swam in the marginal zone of concentration, turned in the normal manner and swam towards the new kathode, where the dehydrating effect of the solution rapidly killed it.

This will be referred to later in discussing the effect of hypertonic solutions.

There is evidently a very close correspondence between the ciliary response of Opalina and Nyctotherus to stimulation with acid and alkaline solutions and to the current respectively, the correspondence being of such a nature as to suggest that on the anodic side of each animal an effect is produced similar to that of contact with acid, on the kathodic side an effect similar to that of contact with alkali. There are differences, of course, but these are of such a nature as may easily be accounted for by the different conditions of experiment.

In the first place it can seldom happen, under the conditions of experiment with test-solutions, that opposite ends of the animal are simultaneously stimulated by alkali stronger than the general medium and by acid respectively.

In the second place it can never happen that such a double stimulus follows the course of the animal as it swims, so that its opposite ends continue to be bathed with acid and alkali. If such an arrangement could be made it is natural to suppose that Opalina, for example, would swim straightforwards with its anterior end always in the acid and its posterior end in the alkali; just as, under the influence of the current it swims straightforwards with its anterior end directed towards the anode, and its posterior towards the kathode.

V. Modification of galvanotaxis by changes in concentration of the media.

Having seen that there is a correspondence between the action of acid and alkaline solutions and that of the current on forms examined in salt solution, it is natural to enquire whether the same holds good of the species examined in more or less pure water. It is at once evident that such is not the case. Jennings[15] found that Paramœcium exhibited a tendency to collect in acid and to swim away from alkali, much like that which I observed in the case of the alkalinated Opalina; but the many observers who have worked with Paramœcium have all

described its marked attraction to the kathode. In Colpidium the attraction to the kathode is as marked, and I have found it to exhibit an attraction to a tube of acid considerably more marked than that shown by Paramœcia in the same solution (Fig. 48).

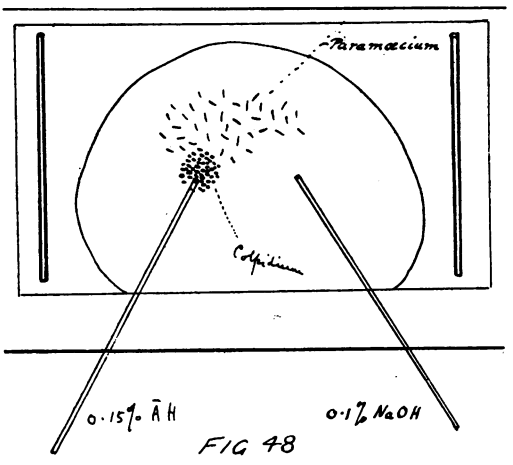


FIG 48

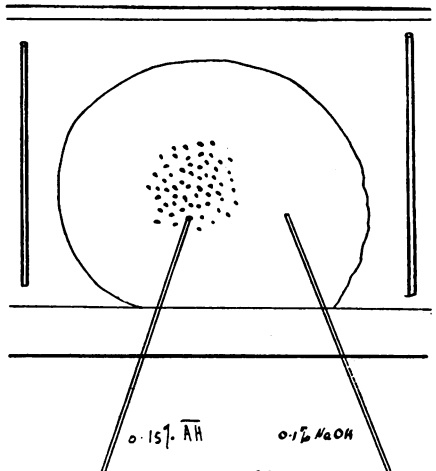


FIG 49

Fig. 48. Chemotaxis of Paramœcium and Colpidium.
Fig. 49. Chemotaxis of Nyctotherus in tap-water.

In these circumstances it is natural to suggest that the difference is due to the medium in which the animals are tested with the current. Nyctotherus and Opalina were therefore tested in ordinary tap-water, and Colpidium and Paramœcium in salt solutions.

The testing of Opalina in tap-water is made difficult by the rapidity with which it dies in such a solution. Vigorous specimens may, however, be kept quite long enough for the application of the test. The Opalinæ are obtained preferably from a stale frog, in which case their activity is greater and their attraction to the anode, in salt solution, more certain than when they are taken from a fresh frog. When taken from a fresh frog a specimen was first tested in salt solution to make certain that it showed the anodic tendency under such conditions. Such specimens were shaken into tap-water, left for about two minutes and tested with a current from 3, 6, 9 and 12 cells. In all cases Opalina then swam directly to the *kathode* at closure of the current. If a weak current was used and allowed to run for

some minutes a tendency to swim away towards the anode appeared, but the death of the animal soon put an end to the experiment.

Nyctotherus lives for many hours in tap-water and retains its activity, though distilled water is rapidly fatal to it. Nyctotherus was shaken into the tap-water of the laboratory, of which the reaction is slightly alkaline, washed repeatedly, and left in clean water for two hours. At the end of this time, tested on the slide with solutions, it showed distinct attraction to the acid (Fig. 49).

Tested with the current it reacted in a manner of which the following record is typical:

EXP. Current from—

3 cells—immediate passage to kathode, with orientation in lines of current. After about two minutes, collection at kathode begins to break up and animals swim one by one to the anode, where ultimately all collect. If current is reversed they now pass, after a latent period of a few seconds, over to the new anode.

6 cells—very similar reaction.

9 cells—very similar reaction, but movement to anode begins later.

12 cells— „ „ „ but movement to anode begins later still, after five minutes' latent period is accompanied by marked transverse orientation and movement to right of current, and takes place very slowly, giving the impression that animals are swimming against a resistance. If, when tendency to move away from the kathode has appeared, current is reduced to three cells, all pass over rapidly to anode[1].

It seems then, that, after a latent period, alkalinated Nyctotherus in water does show the ordinary tendency to the anode, but that this is preceded by a very rapid collection at the kathode, which must presumably be due to some factor which is not present when the process is observed in salt solution. Whatever this factor may be it produces an effect which differs from any of those seen in salt solutions in the rapidity with which it causes the animals to collect at the kathode. It most nearly resembles the collection at the kathode of Nyctotherus after prolonged treatment with acid. If it has any relation to this phenomenon—being due, for example, to a previously developed acidity which the very weak alkalinity of the tap-water was unable to correct—it ought to be accentuated by artificial acidification of the animals.

To test this point Nyctotherus was left for 10 minutes in tap-water

[1] Cf. Carlgren's description of the behaviour of Volvox (p. 293).

coloured with litmus and made acid by addition of several drops of ·2 % HCl. Tested now with the current, Nyctotherus no longer exhibited the very rapid passage to the kathode, but moved slowly to that pole, and with transverse orientation, as in salt solution. This effect cannot be due to a change in the condition of Nyctotherus, since such change as could be produced by stay in this solution has been proved to increase its attraction to the kathode. The conclusion seems irresistible that this effect of adding acid to the water is due to the resulting increase in electrical conductivity. The justice of this conclusion can be tested by the converse experiment of testing in salt solutions forms which have hitherto been shown to exhibit the kathodic attraction when examined in water. Such experiments were made with Colpidium and Paramœcium.

(*a*) *Colpidium* (*? colpoda*). This form is rather delicate and undergoes dehydration too quickly in ·6 % saline to allow a satisfactory experiment to be made. In ·4 % saline it lives for some time and retains its activity, and in this solution the experiments were made. A measured volume of a culture thick with Colpidia was drawn from the jar, and to this, in a watch-glass, was added twice its volume of ·6% saline with vigorous stirring. After a few minutes the stimulation-trough was filled with this mixture and a current passed from six cells. There was still to be observed a trace of that orientation towards the kathode, so marked in this species when examined in water, but the movement was *not* forwards to the kathode, but backwards to the anode, where the Colpidia formed a collection.

(*b*) *Paramœcium aurelia*, of which large well-fed specimens were used, showed considerably greater resistance to the action of salt solutions, and could be examined in ·6 % saline without difficulty. To a measured volume of a Paramœcium culture was added 4 vols. of ·75% saline. In such a solution this large and rapidly swimming form showed an entirely different reaction to that seen in water. Such individuals as, at closure of current, were swimming with the anterior end towards the kathode appeared to move backwards from it for a short distance and then to turn round and swim to the anode—a reaction which recalls in a striking manner the "motor reflex" described by Jennings as occurring when the anterior end comes into contact with an alkaline solution. Those which were swimming towards the anode swam straight on in that direction. The reaction was in no case marked; an individual which had reached the anode often swimming back part of the way to the kathode and then again turning. Further

experiments are necessary before the varieties of response can be classified, but the important points for the present discussion are:

(1) that there is no orientation towards and passage to the kathode as in water;

(2) that the effect of the current is a very weak one;

(3) that the general tendency is to swim towards the anode.

It may be noted that Pearl describes Colpidium as turning sharply from the kathode at break of *current*.

Loeb and Budgett [4], who describe the anodic tendency of Paramœcium in salt solution, attribute it to the habit of swimming backwards which the animal exhibits in such a solution. This I have observed, though the tendency is by no means so constant as I had been led by their description to expect.

For the present it is sufficient to point out that these experiments on Paramœcium and Colpidium confirm the conclusion that nearly all the phenomena described, hitherto, as galvanotaxis are conditioned by the electrical resistance of the fluid in which the experiment is made, disappearing, or being replaced by quite different reactions when the resistance is weakened by the addition of electrolytes.

We now pass to experiments of a different kind, made to determine the effect of increasing the proportion of electrolytes in the solution above that which was found necessary to cause a correspondence between chemotactic and galvanotactic reaction. At present experiments have been made only on Opalina and Nyctotherus. The difficulty of keeping the animals alive for any time in a hypertonic solution is considerable. In solutions of strengths up to 1% NaCl they live for some time, but above that strength dehydration becomes rapid. Nyctotherus, curiously, while very resistant to the effect of hypotonic, is affected almost as rapidly as Opalina by hypertonic solutions. It was found impossible, therefore, to make preparations in solutions of accurately adjusted strength. If the animals were shaken directly into a solution of a strength above 1% NaCl they died very rapidly, and a method was therefore adopted which allows only an approximate determination of the strength, but at the same time enables one to be sure that the solution is hypertonic, and which gives well-marked and characteristic results. The following are the details of the successful method.

Opalina and Nyctotherus were shaken into a watch-glass containing neutral saline and to this was added several drops of ·08 % NaOH. The resulting alkaline solution was left for an hour and then tested in

the trough, to make certain of the anodic collection of both forms. They collected at the anode with about equal rapidity. The trough was now left for 10 minutes in a dish with a shallow layer of 1·5 % NaCl. It was taken out and carefully filled with fresh solution of this strength. Opalina and Nyctotherus, in the alkaline ·6 % saline, were drawn into a fine pipette and allowed to collect by gravitation near the nozzle of the latter. A small drop of ·6 % saline containing a large number of both species was carefully blown into the 1·5 % saline in the stimulation trough. In the middle of this drop they rapidly formed a cluster. A current of 10 cells was now made. On such individuals as remained in the middle of the drop it appeared to have little effect, repulsion from the strong saline being presumably more powerful: occasionally, however, a Nyctotherus, swimming rapidly, would pass the edge of the drop and come into the 1·5 % saline. It immediately turned towards the *kathode* and swam at a moderate pace towards that side of the trough. By stirring with a needle the small drop of ·6 % saline was now completely mixed with the 1·5 % solution filling the chamber, and the animals scattered into the solution. Both Opalina and Nyctotherus showed an unmistakeable attraction to the kathode; Nyctotherus, which had retained its vigour better in the alkaline solution, swimming rapidly to that side of the chamber and forming there a collection. Reversal of current caused movement towards the new kathode.

Experiments were also made with the object of determining, if possible, the point of concentration at which anodic gives way to kathodic attraction. The method was necessarily rather a rough one, the trough being filled with solutions of pure NaCl of varying strengths, Opalina shaken directly from the rectum of a stale frog into the solution, and the current made at once.

The following is the record of a successful experiment, but it must be stated that many experiments were tried with no appreciable result, owing to the rapid death of the animals.

Exp. Upper end of rectum of stale frog, containing many Opalinæ, cut into small pieces, which were shaken successively in salt solutions of increasing strength, the trough in every case being first thoroughly saturated with the solution. Current of 3, 6, 9 and 12 cells used.

In ·6 per cent. NaCl—rapidly to anode with all strengths.

In ·75 per cent. NaCl— „ „ „ „ „ „

In 1 per cent. NaCl—slowly to anode with strong current (9—12 cells) only. Weaker currents no apparent effect.

In 1·2 per cent. NaCl—no apparent galvanotaxis with any strength of current though animals remained fairly vigorous for some time after closure of current.

In 1·3 per cent. NaCl—slight tendency to collect at *kathode* with strong currents.

In 1·5 per cent. NaCl—more marked tendency to *kathode*.

As evidence of the really specific and not merely accidental nature of this phenomenon I may mention that I had a few minutes previously made the same experiment without the precaution of saturating the trough, before each observation, with the solution to be used, contenting myself with washing it in ·6 % NaCl after each experiment. I had then observed a distinct collection at the anode in (so-called) 1·2 % NaCl, and no appreciable reaction in 1·3 %, each solution being weakened by admixture with the fluid wetting the trough. The mere admixture of rectal contents probably weakens the solutions to an appreciable extent, so that the real strength of the solution in which galvanotaxis can no longer be detected lies probably between 1 and 1·2 %, and possibly as low as 1 % NaCl.

In this action of a hypertonic solution we probably have the explanation of the anomaly mentioned earlier. Nyctotherus having swum to the anodic edge of the coverslip probably came into a zone where the solution had by evaporation become hypertonic. As there described it showed, in this zone, a reversed galvanotaxis, swimming now to the *kathode* when the current was made.

VI. Summary and theoretical consideration of results.

We may summarise the results obtained in these experiments as follows:

(1) The five species of parasitic infusoria examined exhibit, in physiological saline, chemotactic reactions to acids and alkalis, which are to a great extent parallel to the galvanotactic response to a constant current, attraction to acid being coincident with attraction to the anode, attraction to alkali with that to the kathode.

(2) Both kinds of response can be modified by treatment of the organisms with acids and alkalis, the course of modification being such that the parallelism is maintained.

(3) Collection in a solution or at an electrode, or repulsion from either, is brought about by certain definite and corresponding alterations of ciliary action.

(4) Examination in a solution containing a very small proportion of electrolytes introduces such modifications into the galvanotactic response as disturb the parallelism to chemotaxis and assimilate the galvanotaxis to that of the ciliates previously examined in water. On the other hand, addition of electrolytes to the water containing such other ciliates eliminates the chief factor in their galvanotaxis, as hitherto described, and produces in their case the parallelism to chemotaxis.

(5) Increase of the proportion of electrolytes above the point necessary to eliminate this factor weakens the galvanotactic response, which at a certain stage of concentration is inappreciable; it reappears as the concentration is further increased, but is now in the opposite direction—attraction to the kathode corresponding to attraction to acid.

In dealing with these results the first point that calls for consideration is the nature of the disturbing element which enters into, and indeed forms in most cases the essential feature of galvanotaxis in *water*. The observations of Birukoff[6] naturally suggest themselves, in which it was shown that the collection at the kathode under such conditions could be imitated by the use of inert particles. Reuss[16] and, after him, Jürgensen[11] described the movement of particles *against* the kataphoric stream and, therefore, to the anode: but Quincke[10] found that, with weak currents, the stream of water, close to the wall of the capillary containing it, carried the particles with it to the kathode, and Weyl[12], under the same conditions as those of Birukoff's experiment, viz., with a thin film of water between a glass slide and a coverslip, and induction-shocks as current, found that the heavier particles only were carried to the anode, the lighter with the stream of water to the kathode. There can hardly be any doubt, then, that under such conditions as those of Birukoff's experiment, viz., with induction shocks and with a thin film of water between glass surfaces, Paramœcia, like other light particles, would be carried to the kathode by the kataphoric stream. But, although the porous ends of the chamber used in most experiments on galvanotaxis will assist in the production of a uniform kataphoric stream, it cannot without further examination be assumed that the behaviour of inert particles will be the same as in Weyl's and Birukoff's experiments, and still less that the galvanotaxis of ciliates in water is entirely due to the same causes. Ludloff[3] attempted to dispose of this view of the physical nature of galvanotaxis by showing that the rate of movement

to the kathode was not proportional to the strength of the current. It must, however, be pointed out that, even on the supposition of a purely physical origin, such a proportional relation could not be expected, since Quincke has shown that there is an antagonism between the kataphoric action on water in contact with glass, etc., and the anaphoric action on particles in contact with water, the latter predominating with strong, the former with weak currents. This of itself might sufficiently account for the decrease in the rate of movement to the kathode with increase of current beyond a certain optimum strength. Again, I have shown that this kataphoric element is eliminated by the addition of electrolytes to the water, as it would be if physical in origin. There is also a suggestive similarity between the orientation of the organisms along lines of current and the similar orientation demonstrated by Faraday[17] and by Weyl in the case of inert, elongate particles. A closer inspection of the phenomenon precludes, however, the acceptance of so simple a physical theory of the nature of this kataphoric element of galvanotaxis in water. It has been shown by Ludloff and by Pearl that the orientation in lines of current is brought about by no merely passive rotation of the animal, but by a definite and constant modification of the ciliary movement. Further, with the strength of current employed in these experiments, and with an open trough, it is impossible, in my experience, to detect a trace of orientating or kataphoric action on dead, or even sluggish infusoria, or on inert particles[1], even in distilled water. In view of the fact that this kathodic galvanotaxis, while dependent on physical conditions such as are necessary for the kataphoric action of the current, has, nevertheless, all the appearance of a truly physiological effect, I would make a suggestion which has not, I believe, hitherto, been put forward. We may suppose that the galvanotaxis in water is a complex of at least two effects: the one, which still remains in salt solution, will be dealt with later: the other is the effect produced by the kataphoric stream of water, which, while it is often, under the conditions of experiment, too weak to produce appreciable effects of purely physical nature, may be strong enough to act as a stimulus to the organism, producing a tendency to swim in the direction of the stream. One cannot suppose that a force tending to drag the organism through the water to the anode would have any different effect, as a stimulus, from a stream of water to the kathode.

[1] Starch grains were used.

Thus this supposition affords an explanation to the observation of Ludloff and others that, with very strong currents, Paramœcium continues to swim violently towards the kathode while dragged back towards the anode by the anaphoric action of the current. It is sufficient to watch the behaviour of Paramœcium, in water contained in a tall jar in which convection currents have been produced, in order to be convinced of its tendency to set itself in the direction of flow and swim with a stream of water. Yet another effect of the current might act as a stimulus producing this tendency to swim towards the kathode. I refer to the endosmotic effects produced in the protoplasm of the animal itself. A swelling at the kathode and shrinking at the anode was described by Du Bois-Reymond[6], in 1860, in a cylinder of egg-white, through which a current was passed. There cannot be much doubt that this purely physical phenomenon, due to the kataphoric effect of the current on the water in the colloid cylinder, plays a large part in the so-called stimulating effects of the current on Rhizopods, and in the shrinkage of such a form as Paramœcium at the anodic and its swelling at the kathodic end, described by Ludloff[3], Loeb and Budgett[4] and others as the effect of strong currents. Carlgren[7] has proved the essentially physical basis of these phenomena by producing them in *dead* Rhizopods and Ciliates. Nevertheless, it would be possible to suppose that this process acted as a kind of mechanical stimulus, producing the swimming to the kathode, were not the latter abolished by addition of electrolytes to the water, which, unless they diffuse quickly into the animal, should not interfere with this physical effect.

We may leave, for the present, consideration of the details of ciliary action in water and turn to the effects of current on infusoria in salt solutions. It has been shown that, under these conditions, there is a close correspondence between galvanotaxis and chemotaxis; but, before considering the possibility of a common interpretation of the two classes of phenomena, the resemblance of the galvanotactic effects to the "electrical coagulation" described by Hardy[13] must be pointed out. According to his observations minute particles of proteid suspended in water move to the anode or kathode according as their reaction is alkaline or acid, the action becoming very slight as the reaction approaches the neutral point and being presumably *nil* when the reaction is absolutely neutral. Moreover, particles collected by the current at the anode there acquire a tendency to move to the kathode. The general result is suggestively similar to those which I obtained

with such a form as Nyctotherus, and the change of reaction caused by stay at one pole recalls the diphasic galvanotaxis of the various species of Balantidium *supra*. There appears, moreover, to be no evidence that the effects on minute particles would not be reproduced in the case of larger masses of proteid, provided that these were kept in suspension, as by ciliary action.

But the view that the phenomena I have described are of such a nature seems untenable for the following reasons:

(1) The exact dependence of anodic or kathodic galvanotaxis on alkalination or acidification was found only in the case of one species[1], in which the chemotaxis showed a parallel dependence.

(2) My experiments were made in a solution containing such a proportion of electrolyte as would probably abolish the physical effect. Hardy found that undialysed proteid showed considerably weaker effects than dialysed.

(3) I have shown that the movements are due to definite actions of the cilia, which again correspond to those produced by chemical stimuli.

(4) There appears to be no explanation, on this theory, of the reversed effect obtained by increase of the proportion of salt beyond a certain point.

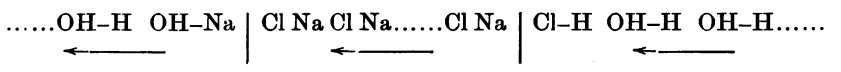
(5) There seems to be no reason why, if non-polarisable electrodes are used, the reaction of particles should be reversed by stay at a pole.

Turning, then, to the possibility of explaining galvanotactic reactions in terms of chemotaxis, we find that such an attempt has already been made, by Loeb and Budgett[4], in the case of Paramœcium stimulated in water. They supposed that alkali is set free where the current leaves the water to enter the animal, acid where it leaves the animal to enter the water. The only evidence for such a view brought forward by its propounders is the supposed similarity between the effects produced at the hinder end of the animal by a strong current, on the one hand, and by immersion of the whole animal in strong alkali (·1 per cent. NaOH) on the other. We may leave out of account the drastic effect of such a solution, which kills the animal in the course of a few seconds, and be content to point out that, if we admit that the body of Paramœcium is composed of sensitive protoplasm, capable of conducting impulses and

[1] It would be possible, however, to explain away such a fact as the kathodic galvanotaxis of Balantidium elongatum after long treatment with weak alkali on the supposition that its protoplasm is normally acid and that the animal is very resistant to an alkaline medium.

contractions, this effect of immersion in alkali will serve equally well, or equally ill, as evidence in favour of the view that alkali is liberated at the *kathodic* or *anterior* end of the animal. The point, however, is not worth further discussion in view of the fact that this appearance of a constricted tip or point ("Zipfel") at the hinder end of the animal has been demonstrated by Carlgren on the dead animal, and is probably a purely physical effect. The possibility of galvanotaxis being due to alkali and acid liberated at the electrodes was discussed by Mouton[5], who decided against such a view. His experiments, however, were made in water, in which case the galvanotaxis is obviously towards a pole where a *repellent* chemotactic effect might be expected. The experiments also differ from most other experiments on galvanotaxis in the important detail that metal electrodes were used. I have attempted in vain to discover evidence of the liberation of acid and alkali with the non-polarisable electrodes used in my experiments. The brush electrodes were dipped into the two limbs of a U tube filled with ·6 per cent. saline, to which phenolphthalein was added, and current from 12 cells was passed for 24 hours without the appearance of a trace of colouring at the kathode. No more effect was obtained when the current was passed in the usual way through salt solution in the stimulation-trough. Further, the action of the current on individuals remote from the poles appears as quickly as on those in the immediate neighbourhood. If the electrodes are dipped into the solution at opposite sides of a watch-glass the effect on individuals in the middle of the glass appears a few seconds after make of the current, long before products liberated at the poles could possibly have reached them by diffusion. We are driven, therefore, to the conclusion that, if the liberation of ions is responsible for these phenomena, it must occur where the current leaves the solution to enter the animal and the animal to enter the solution; it must be, in fact, a case of the polarisation at the common surface of dissimilar electrolytes described by Du Bois-Reymond[19]. Attempts have hitherto failed to detect, by the coloration of the medium with indicators, at which end of the animal acid, at which end alkali, is set free. In the absence of such experimental evidence it will be useful to consider which of the two possible conditions is the more probable. Hermann[20] proved, by experiment, that when a current passes from a weaker to a stronger solution of a given electrolyte there is a liberation of acid at the common surface, alkali being liberated when it passes in the opposite direction. If then we have a layer of stronger between two layers of

weaker solution of alkaline salt, when the current passes, acid will be set free where it enters, alkali where it leaves the stronger solution. Hermann gives a diagrammatic representation of the action, of which the following is a slight modification :—



He uses this observation to explain the fact that, even with non-polarisable electrodes, an acid taste is developed where a current enters the tongue, an alkaline taste when it leaves it [21].

The decision, then, depends on the relative concentration in electrolytes of the protoplasm of Paramœcium and the water which it inhabits. If the proportion of electrolytes in the water is greater, then Loeb and Budgett suppose correctly that alkali is set free where the current leaves the water. Direct evidence on the question is well-nigh impossible : one can only refer to such indications as that a dead Paramœcium absorbs water from the medium, with the consequence that the protoplasm swells up. The same is true of the infusoria parasitic in frogs' intestine when they die in ·6 per cent. saline, albeit such saline appears during life to cause slight shrinkage rather than swelling. On the supposition that the proportion of salt in the protoplasm is greater than that in the water my results at once become intelligible. Under these conditions acid would be set free at the anodic, alkali at the kathodic end of the animal, and this double chemical stimulus would follow the animal as it swam. We have seen that the results are just what might be expected on such a supposition. An Opalina which became deflected from the line of current would have alkali set free along one edge, acid along the other, with the result that it would tend to return to the anode-pointing position. In this position the waves of ciliary motion should pass straight backwards, as in fact they do, while make of the current when the animal points to the kathode should cause forward striking of the cilia, succeeded by rotation into the anode-pointing position, which is also actually the case. Such differences as do appear between the two types of reaction are such as might be predicted from the facts that the animal can swim out of range of the solution diffusing from a capillary, while it cannot swim away from the stimulus caused by the current.

Such a supposition also accounts for the effect obtained by increasing the proportion of salt in the solution. When the proportion of salts becomes equal to that in the protoplasm no reaction should be observed.

A strength of solution was actually found in which Opalina appeared immune to the current. Further increase should cause liberation of alkali at the anodic, acid at the kathodic end, and we have seen that such increase caused kathodic galvanotaxis of Opalina and Nyctotherus in the acid-seeking condition.

Returning to the ciliary action in the forms examined in water, Ludloff[8] and Pearl[8] have described, and I have myself seen, a double reaction quite different to anything produced by solutions of acid and alkali. Here it may be again pointed out that the conditions are different. Not only on the ideas suggested above is the stimulation with acid and alkali simultaneous and persistent, but there is another element in the stimulus of such a nature that it keeps the anterior end directed to the *kathode*. The effect of momentary contact of Paramœcium with an alkaline solution is that reversal of the cilia drives it backwards out of reach of the solution, and there seems nothing unnatural in the supposition that *continuous* stimulation of the anterior end with alkali would cause a continuous forward stroke of the cilia on the anterior half of the animal. We can imagine, then, that the ordinary galvanotaxis of Paramœcium is a complex of two opposing factors—the rheotaxis caused by the kataphoric stream, which causes orientation in the lines of current with the anterior end towards the kathode and augmentation of the normal backward ciliary stroke, and the chemotactic effect of alkali set free at the kathodic side and acid set free at the anodic side of the animal, which would cause, in a kathode-pointing individual, recession from the kathode, caused by forward striking of the cilia, followed by rotation into the anodic-pointing position. In water the former is the predominant factor, keeping the animal in the kathode-pointing position and so augmenting the ciliary action in the hinder part of the animal that the forward stroke of cilia at the anterior end, which may be regarded as the effect of alkali there set free, is overpowered, and the animal moves straight to the kathode.

With the use of stronger currents the purely physical kataphoric effect probably assists the passage to the kathode; and with still stronger currents the anaphoric effect may, as in Quincke's[10] experiment, become predominant. The *physiological effect—i.e.* the effect on the ciliary action—should, as already pointed out, be unaltered or intensified by this action: and Ludloff[8] has shown that the passage of the animal to the kathode may be slowed, or stopped, or it may even be dragged backwards to the anode, while the cilia still strike backwards on its posterior, forwards on its anterior half.

A difference must here be noted between the two effects, viz. that the rheotactic effect appears more rapidly and disappears more immediately with closure or break of the circuit. Paramœcium or Nyctotherus in water turns to the kathode at make of the current with a scarcely perceptible interval. In salt solution there is a latent period of some seconds before the effects appear, and they fade away, rather than cease suddenly, at break. It may be expected, then, especially if the second stimulus is of chemical nature, that, after break of current, it will be for a brief interval effective, uncomplicated by the rheotactic element. Pearl [8] describes a *break* stimulus, viz. a quick rotation from the kathode. This is exactly what one would expect in the case of an animal, held by other influences towards a repellent stimulus, when the constraining force was suddenly removed.

Addition of a small proportion of electrolyte may so weaken the rheotactic factor that, though the animal is still kept by it in the kathode-pointing position, the reversed cilia at its anterior end now drive it backwards to the anode. Such a condition was obtained when Colpidium was examined in ·4 % NaCl. Further addition may completely abolish the rheotaxis, in which case the galvanotaxis should consist entirely of chemotactic factors; and the experiments in ·6 % saline give results which are readily interpreted on such a theory.

A macroscopic inspection of the phenomena provokes the natural suggestion that attraction to a pole or a solution means inhibition on the side of the animal affected by either, while repulsion is brought about by unilateral stimulation. Such a theory was propounded by Verworn [2] as regards current action, and further developed by Loeb and Budgett [4]. The latter observers attributed the backward stroke of the cilia on the anodic part of Paramœcium to alkali there liberated, the forward stroke on the kathodic part to liberation of acid. A comparison with the observed effects of solutions on Paramœcium (Jennings) shows that acid leaves the normal backward stroke unchanged while the first effect of alkali is to cause forward striking of the cilia; so that, on their supposition, exactly the reverse of the effects observed might be expected. But, in any case, a study of the details of ciliary action, in salt solution, under the influence of either stimulus, shows quite clearly that, as was maintained by Schenck [23] in a criticism of Verworn's results, the action is not localised or even unilateral, but is produced by a coordinated and, in some cases, complicated reflex involving all the body cilia. A reference to the description of the ciliary action of alkalinated Opalina in contact with

solutions will make it clear that the effect of unilateral contact with acid is no more inhibitory than that of unilateral contact with alkali. Both produce a coordinated reflex involving the cilia of the whole body, so that if the animal could be stimulated simultaneously with acid or alkali on opposite sides it would be practically impossible to discriminate the part played by each stimulus in the resulting ciliary reflex, one effect being in some sense the complement of the other. Equally impossible would it be in any particular case of galvanotaxis to attribute to changes at one pole or other of the animal the observed changes in ciliary motion.

The correspondence between the effects of electrical and chemical stimuli extends even to the effect of varying the strength of the stimulus. In the case of alkalinated Opalina and Nyctotherus it was pointed out that acid above a certain strength had a repellent effect similar to that of alkali; if a current of more than a certain strength is used both poles seem to have a repellent effect, the animal swimming transversely to the lines of current towards the sides of the chamber where the density of current is less. The three species of Balantidium, again, showed a diphasic reaction to stimuli of both kinds.

For the above theory no more is claimed than that it affords a convenient means of summarising the results of these experiments. It emphasises the fact that many of the phenomena of galvanotaxis show a close correspondence with certain chemotactic phenomena; so close that the possibility of a common explanation is at least worth consideration. How far the theory in the form given above is adequate and justified, further experiment must determine. In the absence of present opportunity for such further experiment it has seemed desirable to publish the results obtained, with such proximate explanations as have suggested themselves.

I have pleasure in recording my indebtedness to Mr W. B. Hardy and to Dr Langley for much kind help and advice.

Addendum. In a recent paper Pütter[24] has described, in Stylonychia and Urostyla, a reaction very similar to that I have given on p. 314. Individuals adhering by thigmotaxis to the glass bottom of the chamber, or to the surface film, are described as exhibiting, with moderate or strong currents, a transverse orientation, with the kathode to the animal's left, precisely similar to that which I had observed in Nyctotherus. Free-swimming individuals exhibited the ordinary kathodic galvanotaxis of ciliate infusoria examined in

water, which will be discussed later. As mentioned above, Nyctotherus is conspicuous for its tendency to swim with its side applied to the floor of the chamber.

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